GENETIC AND ECOLOGICAL PERSPECTIVE OF THE RECOVERY OF CORAL REEFS IN PALAU

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DEDICATION

To my mom and dad, who, in the past five years have learned as much about population genetics and reef resilience as I have.

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ABSTRACT

Connectivity plays a fundamental role in structuring populations. Understanding the connectivity process has important implications for the conservation of marine organisms, particularly in the design of marine protected areas. However, tracking pelagic larvae in a marine environment is challenging and there is still a lack of field data to incorporate connectivity into marine conservation planning. This research uses high throughput microsatellite genotyping of the coral *Acropora hyacinthus* to characterize population genetic structure of the reefs of Palau after the 1998 bleaching event in order to understand the processes driving the recovery of the reef and connectivity of populations and to provide management recommendations. The results of the study indicate that Palau did not recover from a pulse event of long distance dispersal from Yap, 452 km away, but from surviving coral colonies (Chapter II); that populations of *Acropora hyacinthus* are self-seeding, creating a mosaic of genetic neighborhoods around Palau (Chapter III) and that short distance dispersal is responsible for the genetic structure of the population of *Acropora hyacinthus* while genetic neighborhoods can explain patterns of chaotic genetic patchiness at a larger scale (Chapter IV). The study also finds that the coral cover of the reefs of Palau recovered at different rates for deep and shallow sites and for different locations but without any shift towards an algae dominated reef (Chapter V). These results, and the prediction that Palau will be impacted by more frequent thermal stress, support the recommendations that authorities should increase conservation efforts locally rather than at a regional level with priority given to managing reefs outside MPAs and to extending the areas of protection (Chapter VI). Overall, the findings of this study highlight that, contrary to the long-lived paradigm that marine populations are structured by large-scale connectivity, short distance dispersal can dictate the genetic differences coral populations.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

CHAPTER I. INTRODUCTION

Connectivity, defined as the exchange of individuals among geographically separated populations, plays a fundamental role in structuring populations. It influences population dynamics and impacts the long-term survival of communities (Roughgarden et al. 1988; Hanski 1999) and maintains the diversity of the genetic pool by constantly adding new individuals (Wright 1949) increasing the potential of populations to adapt to environmental changes (Soulé and Mills 1992, 1998). For organisms with sessile or sedentary adults such as terrestrial plants, marine invertebrates and fish, connectivity takes place during the early life stage with the dispersal of propagules or pelagic larvae. In terrestrial plant communities, in the majority of cases, dispersal is limited to short distances (Willson 1993; Nathan et al. 2008) and barriers to dispersal are often visible (Holderegger and Wagner 2008). For marine organisms reproducing via pelagic larvae, a long lived paradigm is that there are few barriers to dispersal in a fluid environment enabling pelagic larvae to travel large distances leading to populations that are well mixed with very little genetic differences between regions (Cowen et al. 2007).

The problem with studying connectivity of marine organisms is that it is extremely difficult to track minuscule planktonic larvae in the marine environment. One approach has been to develop oceanographic models that predict larval pathways, however, there has been very little direct field data to test them (Gawarkiewicz et al. 2007). Population genetics and molecular ecology techniques tackle the question from a different angle and use adult populations to indirectly infer where larvae are travelling and settling (Hellberg et al. 2002; Hedgecock et al. 2003). Recent work in both fields are challenging the assumptions that marine population are well connected (Cowen 2002; Gaines et al. 2007; Jones et al. 2009; Weersing and Toonen 2009). Studies are finding more genetic structure between populations at regional and local scale than expected.

The scale of connectivity and the mechanisms that drive dispersal between populations have important implications for the conservation of marine organisms. Marine ecosystems and biodiversity are increasingly impacted by diverse threats, in particular over-harvesting, pollution and climate change (Hixon et al. 2001; Halpern et al. 2007; Worm and Lenihan 2013). One of the primary tools for the conservation of marine

organisms worldwide is marine protected areas (MPAs) (Lubchenco et al. 2003). Originally designed, among other goals, to protect biodiversity and fisheries resources, recent increase in thermal stresses and their impacts on marine communities has lead to the review of MPAs to help maintain ecosystem resilience by spreading risk (IUCN 2008; Walton et al. 2014). There has been a particular focus on coral reefs (Selig et al. 2010) and conservation agencies such as The Nature Conservancy have been developing guidelines on how to design networks of MPAs to increase resilience of coral reefs (Marshall and Schuttenberg 2006; McLeod et al. 2009; Sale et al. 2010; Magris et al. 2014). The principle behind these networks is based on the hypothesis that reefs with high mortality depend on connectivity to other more healthy reefs for re-seeding. Resilient MPA networks are designed to ensure that areas that are resistant to climate change are connected to those that are more vulnerable to increase the likelihood of reef survival (Hughes et al. 2003).

The central goal of this dissertation is to contribute to the understanding of connectivity of marine organisms and contribute to management guidelines of the design of resilient networks of marine protected areas. Using high throughput microsatellite genotyping of the coral *Acropora hyacinthus* to characterize population genetic structure of the reefs of Palau after the 1998 bleaching event as a case study, I will address the following questions:

- Does long distance dispersal influence demographic processes such as coral reef recovery?
- At what scale does dispersal define the structure of coral populations?
- How does non-equilibrium between populations influence our interpretation of population genetic structure at different scales?

All four chapters of this dissertation are additionally linked by a management question: how do you define a resilient coral community and how is it connected to the rest of the reef? Chapter 2 challenges the hypothesis based on an oceanographic model that a pulse event of long distance dispersal of coral larvae from Yap, 452 km away from Palau, enabled the reefs of Palau to recover. Chapter 3 takes a closer look at the population

structure of *A. hyacinthus* around the reefs of Palau to understand the mechanisms of recovery and identify areas that act as sources of larvae to recolonize the reef. I use kinship as a measure of population structure to test for self-seeding. Chapter 4 compares four scales of population structure to infer gene flow. I use both measures of population structure (*F*-statistics) and measures of individual genetic differences (kinship coefficients). *F*-statistics measure population structure with the assumption that populations are in equilibrium, whereas kinship coefficient does not have such assumption and can reveal population disequilibrium. Chapter 5 focuses on the ecological recovery of the reefs if Palau. I re-analyze monitoring data as a time series of benthic cover to find factors that enable recovery. Finally, in chapter 6, I provide an overview of how this work advances the question of connectivity for marine organisms and summarize guidelines for the design of resilient marine protected areas.

CHAPTER II. POPULATION GENETIC STRUCTURE BETWEEN YAP AND PALAU FOR THE CORAL *ACROPORA HYACINTHUS*

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Abstract

Information on connectivity is becoming increasingly in demand as marine protected areas are being designed as an integral part of a network to protect marine resources at the ecosystem level. Larval dispersal and population structure, however, remain very difficult to assess. Here, I tested the predictions of a detailed oceanographic connectivity model of larval dispersal and coral recruitment within Palau and between Palau and Yap, which was developed to support the review of the existing network of marine protected areas in Palau. I used high throughput microsatellite genotyping of the coral *Acropora hyacinthus* to characterize population genetic structure. Pairwise F'_{ST} values between Palau and Yap (0.10), Palau and Ngulu (0.09) and Yap and Ngulu (0.09) were all significant and similar to pairwise F'_{ST} values of sites within Palau (0.02 to 0.12) and within Yap (0.02 to 0.09) highlighting structure at island scale and indicating that recruitment may be even more localized than previously anticipated. A bottleneck test did not reveal any signs of a founder effect between Yap and Palau. Overall, the data supports the idea that recovery of *A. hyacinthus* in Palau did not come exclusively from a single source but most likely came from a combination of areas, including sites within Palau. In light of these results there seems to be very little connectivity around the barrier reef and management recommendation would be to increase the number or the size of MPAs within Palau.

Introduction

Over the past forty years, the United Nations Environment Programme (UNEP) Regional Seas Convention and Action Plans has been encouraging countries to work together to protect marine resources at a regional scale (Johnson et al. 2014). In the last decade, this approach targeting the protection of coral reefs has been adopted through initiatives such as the Micronesia Challenge, the Coral Triangle Initiative on Coral Reefs, Fisheries and Food Security, and the Caribbean Challenge Initiative. Island nations are joining forces to protect their near-shore resources, not only at a national scale but also by considering the integrity of their ecosystems at a regional scale. As a consequence, managers have had to scale up existing conservation strategies to adapt them to larger areas. One approach has been to develop Marine Protected Area (MPA) networks as a tool to address the conservation of coral reefs across borders. Designed to maintain connectivity at the scale of ecosystem processes, MPA networks build resilience by spreading risk in the case of localized disasters, climate change, failures in management or other hazards, and overall, help protect biodiversity and fisheries resources (IUCN 2008; Walton et al. 2014). These networks also allow for building upon existing MPAs by maintaining structures that are already in place and enhancing resilience to these areas (McLeod et al. 2009).

As a member of the Micronesia Challenge, Palau is one of the nations to have adopted resilient MPA networks as part of their strategy to effectively conserve at least 30% of the near-shore marine resources across Micronesia. Palau is an archipelago that suffered heavy bleaching mortality during the 1998 El Niño bleaching event and in response to that mortality, established a network of marine protected areas to encourage reef recovery. The initial MPAs, however, were not designed with the specific purpose of maintaining connectivity between reefs and there has been a national effort to review the design of their MPA network to provide regional resilience to both local and global scale stressors (Golbuu et al. 2005).

In practice, implementing MPA networks that are interconnected and thus resilient faces the challenge of understanding connectivity of marine communities. Many marine organisms reproduce via minute pelagic larvae that are difficult to track and the barriers and drivers influencing their dispersal are not always obvious, making information on connectivity difficult to determine (reviewed in Levin, 2006; Cowen & Sponaugle, 2009). To address such issues,

researchers have taken two main approaches: first, developing oceanographic models of the dispersal of particles forced with physical data (e.g., wind and tides) or second, indirect/direct tagging via molecular (i.e., DNA) or chemical markers (i.e., otolith/statolith chemistry) to study gene flow between populations (Hellberg, 2007; Cowen & Sponaugle, 2009).

In the most detailed oceanographic connectivity model for Palau to date, Golbuu et al. (2012) incorporated reef architecture at 500m scales with over 30 years of oceanographic data to predict larval dispersal and coral recruitment both within Palau and between Palau and Yap, a neighboring Micronesian archipelago 452km from Palau. They concluded that Palau recovered quickly after the mass 1998 bleaching mortality due to a pulse event of larval dispersal from Yap in 1999. The model also predicted considerable local retention at all sites in the Palau archipelago on a short time scale (<3 days), which changed at longer time scale (>3 days) with the northern lagoon showing the most flushing. The model also indicated a directional dispersal from densely populated areas (i.e. the southern lagoon) to less densely populated areas (i.e. the northern lagoon). Based on the results of their dispersal modeling, Golbuu et al. (2012) recommend building a network of MPAs at a regional and national scale that would link Yap and Palau, and Palau's northern and southern reefs.

A common alternative to oceanographic model to estimate rates, distances and patterns of dispersal is genetic analysis. Microsatellites have been the marker of choice, using fragment analysis to create genotypes based on length. This method, however, has several issues, including the problem of homoplasy, which can reduce allelic diversity in populations and inflate estimates of gene flow when mutation rate is high (Selkoe and Toonen 2006). Sequencing microsatellites can resolve this issue by allowing scoring microsatellites according to sequences, yet very few studies have tried this approach. Here, I use high throughput microsatellite genotyping of the coral *Acropora hyacinthus* to characterize population genetic structure between Yap and Palau to test the predictions made by the dispersal modeling of Golbuu et al. (2012).

Methods

Study species

Acropora hyacinthus was an abundant tabular coral found on the reef slopes of Palau prior to the 1998 bleaching event. During their 2001 assessment, Bruno et al. (2001) estimated a near complete loss of this coral in the areas they sampled. By 2005, Golbuu et al. (2007) observed that the same species was dominant in the shallow reef slopes, raising the question of where the larvae originated from to allow for such a successful recovery and thereby making *A. hyacinthus* and ideal candidate to study coral connectivity.

A. hyacinthus is usually found between 3 and 10m deep on barrier reefs and is readily identifiable by the rosette formation of its calices (Fig. 2.1). *A. hyacinthus* is a hermaphrodite broadcast spawning coral that produces feeding larvae (Toh et al. 2013) with a pelagic larval duration of approximately 90 days under laboratory conditions (Márquez et al. 2002). Little is known, however, about the pelagic duration or swimming behavior of larvae in the field, which makes realistic incorporation of biological parameters into oceanographic models difficult (Paris et al. 2007; Woodson and McManus 2007).

Sampling locations and methodology

In May 2012, three sites on the outer barrier reef of Palau were sampled at a shallow depth (<10m) using SCUBA (Fig. 2a, 2b). Sites were selected to represent a range of habitats, exposures and management categories found on the barrier reef: S17 "West Palau" within a fully protected no-take area on the west side, S20 "North Palau" at the tip of the northern lagoon in a less strictly protected area and S24 "East Palau" on the east coast in a reef impacted by anthropogenic stressors (Golbuu et al. 2012). At each of these three sites, 48 colonies of *A. hyacinthus* were collected haphazardly in a 4 x 200m belt transect, for a total of 144 colonies from Palau. One small branch tip (<1cm) was cut and preserved in salt-saturated DMSO at room temperature (Gaither et al. 2010b). In addition, a total of 132 samples were collected from three different sites around Yap, and another 46 colonies were sampled from a single site at Ngulu as described in Davies et al. (2015, Table 2.1, Fig. 2.2a, 2.2c, 2.2d)). Importation was permitted by the Convention on International Trade in Endangered Species of Wild Fauna and Flora permit #FW 12-091.

DNA extraction and sequencing

For colonies from Palau, Genomic DNA was isolated following the DNeasy 96 Blood & Tissue Kit (Qiagen, Valencia, CA, USA) protocol. Two sites with 48 colonies each were extracted on a 96 well plate. For each colony, a $2mm^2$ piece of coral from the tips of one branch was ground and incubated overnight at 55° C in 180 µl of Qiagen Lysis buffer and 20 µl of Qiagen Proteinase K (600 mAU/ml). DNA was eluted in 200 µl of PCR grade water, with 100 µl frozen until amplified and 100 µl saved for future use at -20^{o}C. A sample of 50 DNAs was quantified on a SpectraMax (Molecular Devices, Sunnyvale, CA, USA) absorbance microplate reader using Accuclear Ultra High Sensitivity quantitation Kit (Biotium, Hayward, CA, USA). Quantities of DNA were very similar for all colonies, ranging from 5 to 10ng/µl. The DNA of colonies originating from Yap and Ngulu were isolated according to Davies et al. (2015).

Eighteen microsatellite loci developed by Wang, Zhang, and Matz (2009) were selected for this study (Appendix1). I designed short tags according to Faircloth & Glenn (2012) to create 48 unique colony IDs on the forward primer for each microsatellite locus (Appendix 2). Polymerase chain reactions (PCRs) for each colony were performed in a 25 µl reaction volume containing 12.5 µl of MyTaq™ Red Mix (Bioline, Taunton, MA, USA), 1 µl of each forward and reverse primer at 5µM, 1 µL of genomic DNA (5 to 10 ng/µl) and 9.5 µl of water. Thermal cycling followed a touch-down protocol with an initial denaturation at 95° C for 3 min followed by 20 cycles of 95° C for 15 sec, 63 - 55° C for 15 sec (annealing temperature reduced 0.4° C each cycle), 72° C for 30 sec followed by 20 cycles of 95° C for 15 sec, 55° C for 15 sec, 72° C for 30 sec with a final elongation at 72° C for 3 min.

Two µl of each uniquely barcoded amplicon were pooled by site for subsequent library construction and sequencing. The pooled samples were concentrated by reducing the total volume using Amicon Ultra 0.5 mL centrifugal filters (EMD Millipore, Darmstadt, Germany) and cleaned using Agentcourt Ampure XP (Beckman Coulter Inc., Brea, CA, USA) to eliminate traces of dye and unincorporated dNTPs and primers. Genomic libraries were generated using the KAPA Hyper Prep kit (Kapa Biosystems, Wilmington, MA, USA). A unique Illumina adaptor (Illumina Inc., Hayward, CA, USA) was ligated to each pool of individually barcoded amplicon samples, creating a site-specific tag (ID) and generating the following unique structure:

siteID-colonyID-FWDprimer-flankingregion-tandemrepeats-flankingregion-RVSprimer. Libraries were sequenced on an Illumina MiSeq at the Hawai'i Institute of Marine Biology (HIMB) Evolutionary Genetic core facility. Per individual genotype information is available at DRYAD doi:10.5061/dryad.m4q9f.

Bioinformatics (Appendix 3)

Low quality trimming

Illumina adaptors and barcodes were removed and sequences were demultiplexed by site through the Illumina MiSeq Reporter (MSR) software (Illumina, Inc). Forward and reverse sequences were merged using PEAR (Paired-End reAd mergeR) (Zhang et al. 2014). Sequences were then further demultiplexed according to primer and colony barcodes using INTEGROOMER (unpublished, http://courge.ics.hawaii.edu/inte/groomer/). Low quality sequences and reverse primers were trimmed using TRIMMOMATIC (Bolger et al. 2014). Cleaning resulted in sequences consisting of flanking region, tandem repeats and flanking region. Identical sequences were then collapsed into unique sequences and counted (= depth; the number of times a given sequence was repeated in the library).

Filtering

A set of filters developed in PYTHON (https://github.com/annickcros/Ahyacinthus-filters.git) was then applied to the sequences to eliminate PCR and sequencing artifacts. A length filter was applied to eliminate sequences that were less than 15 base pairs and sequences that were greater than 190 base pairs. This maximum length was determined by adding 4 additional repeats to the longest microsatellite sequence in the data set (Appendix 1). The file with the rejected sequences was checked by eye. None of the sequence rejected were true microsatellites. Two depth filters were applied to the sequences of the entire dataset, which were pooled by locus. The first eliminated any sequences with fewer than 10 reads the second eliminated any sequence that was present in less than two individual colonies regardless of sequencing depth (see below).

Scoring

To score microsatellite alleles, for each colony at each locus, the sequence with the highest depth was initially selected. Any sequence that had a depth greater than half of the depth of the most frequent sequence was also selected. This approach resulted in colonies with one (homozygote), two (heterozygote), or in some cases, more than two alleles per locus (see below).

More than 2 alleles

After filters and scoring, there were still a large number of colonies that had more than two apparent alleles among the sequence reads (i.e., multiple sequences were represented at least half as often as the most common allele in the amplicon library). These alleles typically varied either by a single nucleotide difference or by an indel in the repeat region, and could have derived from somatic mutations, individual chimerism, PCR or sequencing errors, or some combination of these factors.

For colonies in which more than two alleles passed the initial filters, I selected the two alleles used for analysis in two different ways. In both cases, I first selected the sequence with the highest depth was selected as the first allele. The second allele was then selected either by: 1) the allele with the next highest sequencing depth (selection by depth), or 2) at random from all the available alternative choices (random selection). In a few cases of selection by depth, there were multiple alternative alleles that were equally frequent, therefore the second allele was selected at random from among the tied second-highest depth reads. Random selection with replacement of the second allele was repeated 10 times to generate 10 independent files.

Splitting Flanking Region Sequences and Simple Tandem Repeats

I separated simple tandem repeats (STR) from their flanking regions using EMBOSS: ETANDEM (Rice et al. 2000) and created genotypes based on the STR length.

GENODIVE files

Datasets were converted to GENODIVE v. 2.0b27 (Meirmans 2009) file format. Individual genotypes were created using two different methods. First, I used sequence length similar to peak calling in a microsatellite fragment analysis, such that all sequences of the same length, regardless of underlying sequence variation, would be scored as the same allele (sequence length). Second, I identified alleles by their sequence (ID) so that only two exactly identical alleles had the same ID, whereas alleles with the same length but differing in nucleotide composition would have different IDs (unique ID).

Only loci with less than 15% missing data and colonies with less than 35% missing data were used for the analysis. The final dataset analysis was carried out on 11 loci (Appendix 4). The

final number of colonies for each locus for each site varies between 37 and 48. The number of alleles and allelic richness is reported in Table 2.2.

Comparison of datasets

To compare the different datasets created by alternate strategies of filtering and scoring, I looked for bias in population differentiation using AMOVA in GENODIVE to calculate F_{ST}, F'_{ST} and F_{IS} and corresponding significance values among and within populations. Indices of genetic diversity, including observed heterozygosity (H_O), expected heterozygosity within populations (H_E), corrected total heterozygosity (H'_T) and Nei's corrected fixation index G'_{ST}, were used to quantify the population diversity (Table 2.2) and check for any potential bias between datasets created using different strategies.

Analysis

Population differentiation

I carried out an AMOVA to calculate population differentiation by island groups. Using GENODIVE v. 2.0b27, I carried out two pairwise differentiation tests (20,000 permutations). The first was among islands. The second pairwise differentiation tests were carried out among all seven sites and exact tests of population differentiation (100,000 steps) were carried out using GENEPOP v. 4.4.3 (Raymond and Rousset 1995; Rousset 2008) to report the *p* value of the pairwise F'_{ST} (Table 2.4).

Bottleneck

To test the hypothesis that *A. hyacinthus* on Palau has recovered from a pulse event of larval dispersal following the 1998 bleaching mortality, I looked for evidence of a recent bottleneck or founder effect using BOTTLENECK 1.2.02 (Piry et al. 1999). In populations that have experienced such an event, rare alleles are the first to be lost, lowering the mean number of alleles per locus. Heterozygosity is less affected, however, producing a transient excess in heterozygosity relative to that expected given the resulting number of alleles (Cornuet and Luikart 1996). I used the graphical test from Luikart et al. (1998) based on a mode shift away from an L-shaped distribution of allelic frequencies to assess whether evidence of recent population bottlenecks could be detected, which is most appropriate for these type of data (Chaïr et al. 2011). Because I scored fewer than 20 microsatellite loci, I used the Wilcoxon signed-rank test (10,000 iterations)

using both a two-phase (TPM incorporated 70% stepwise and 30% multistep mutations) and an infinite allele (IAM) mutational model.

Population structure

A Principal Component Analysis (PCA) was performed in GENODIVE v. 2.0b27 (Meirmans 2009), both on individuals and on sites using a covariance matrix with 10,000 permutations. The graphs were plotted in EXCEL (Microsoft, Redmond, Washington, 2010, Fig. 2.3 and Fig. 2.4).

Results

Comparison of datasets

I detected no evidence that alternate strategies of filtering and scoring showed bias in our inference of population differentiation (Table 2.3). The overall F_{ST} and F'_{ST} values as well as the indices of diversity were almost identical for the 10 datasets generated. Genotypes with alleles identified by sequence length gave slightly lower F'_{ST} and heterozygosity because length masks some of the underlying sequence variation relative to unique IDs, reducing the mean within population heterozygosity and F'_{ST} (Hedrick 2005; Jakobsson et al. 2013). Selecting the second allele by depth biases against within population allelic diversity resulted in slightly higher F_{ST} and F'_{ST} values and lower F_{IS} values. As a conservative approach, I selected the first dataset generated by randomly selecting the second allele when there were more than two choices (random000_len). In each case, however, the differentiation among sites was significant ($p<0.001$), and there are no cases in which inferences would differ because of this variation in magnitude. Because the different strategies for filtering reads produce qualitatively similar results in all cases I believe that the approach of selecting the second allele does not bias our interpretations.

Population differentiation

Population differentiation was significant among all sites, with a global $F'_{ST} = 0.11$. The pairwise differentiation test between islands show that F'_{ST} values between Yap and Ngulu (0.09), Palau and Ngulu (0.10) and Yap and Palau (0.09) were similar and all comparisons were significant ($p < 0.01$). The exact test between sites (Table 2.4) shows that both within and among islands, all comparisons are significantly different between Yap, Palau and Ngulu. For example, on Palau, site S24 shows the most differentiation with site S17 (012), which is of the same magnitude as comparisons among islands above. I calculated a second measure of genetic differentiation,

Jost's D which gave the same differences between sites (Appendix 5).

Bottleneck

The graphical test for bottlenecks which does not require data to be in Hardy-Weinberg equilibrium, is robust to a small number of loci (fewer than 20), and detects bottlenecks that occurred within a few dozen generations (Luikart et al. 1998). All of the sites had normal L-shaped distributions and the test showed no evidence of bottlenecks for the sites around Palau.

Population structure

All samples are distributed more of less evenly along the first and second axis with a slight partition along the first axis such that Palau and Yap sites each cluster together (Fig. 2.3). Very little of the overall variation is explained, with the first axis explaining only 7.2% and the second axis explaining an additional 5.7% of the variation.

When individuals are grouped by sampling location, sites partition along the first and second axis of the PCA into three quadrants, with Ngulu site S28 standing alone, Palau sites S17, S20 and S24 in the left quadrant and Yap sites S27, S29 and S30 in the right quadrant (Fig. 2.4). This PCA accounts for 43% of variation along the first axis and the second axis explains an additional 21% of variation.

Discussion

Connectivity features as a key component of the design of networks of MPAs to increase resilience of both habitats and resources. Larval dispersal and population structure, however, are difficult to measure in the marine environment and different approaches may convey different results to managers. Here, I tested the predictions made by the oceanographic model developed by Golbuu et al. (2012) using high throughput microsatellite genotyping of the coral *A. hyacinthus* and found no evidence that Yap was a significant source of larvae for the recolonization of *A. hyacinthus* after the 1998 bleaching event.

Microsatellite sequencing

Genotyping microsatellite loci using high throughput sequencing has become cheaper and faster than traditional fluorescent fragment length analysis. To our knowledge, however, there have been very few published papers using this technique (Roberts et al. 2004) and no standardized

protocol has yet been developed to obtain the best results. The main challenge with sequenced microsatellites is to define a genotype for each colony. There were cases where regardless of the objective filtering criteria applied, more than two alleles were possible, yet corals are diploid organisms. To ensure that our results were robust to decisions about how to select among alternate possible alleles, I tested different methods of allele selection, including selecting two alleles at random from among all sequence variants within an individual. I find that the data are robust to filtering and allele selection criteria, because although the exact values differed, with consistently lower values for selection by depth, none of the indices of heterozygosity, H_O, H_E, G'_{ST}, or genetic structure, including F_{ST}, F'_{ST} and F_{IS}, fundamentally changed by using different approaches. This can be explained by previous findings that the greatest portion of the structure and diversity is driven by the most common alleles in the population (Selkoe and Toonen 2006; Toonen and Grosberg 2011). This is consistent with our data because neither of the selection criteria impacted the most common alleles in the populations. This robust finding indicates that scoring microsatellites using high throughput technology can give consistent and reliable results that, being based on the underlying sequence rather than length polymorphisms, can allow comparisons of the repeat motif itself and variable flanking regions and be combined reliably among labs and studies. The selection of alleles among several possibilities, however, may prove problematic for applications such as parentage analysis where the genetic pool for parentage assignment will be affected and could lead to false parentage exclusion.

Connectivity between Palau, Yap and Ngulu

Golbuu et al. (2012) show oceanographic connectivity between Palau and Yap, and argue that the reefs of Palau recovered surprisingly quickly from the mass mortality following the 1998 bleaching event due to a pulse of coral larvae from Yap (including the atoll of Ngulu) in 1999. Bruno et al. (2001) describe populations of *A. hyacinthus* in Palau as having suffered virtually 100% mortality in areas that were surveyed. Given the generation time of *A. hyacinthus* (Wallace 1985), I would expect roughly three generations in the 14 years between this mortality and our sampling in 2012. If Palau had recovered from a pulse recruitment from Yap after such a widespread and dramatic loss, I would expect evidence of a bottleneck in the recovering population, and should be able to observe a significant founder effect such that Palau would contain a strict subset of the total diversity of Yap and Ngulu from which it was recolonized. Instead, I did not observe signs of a bottleneck, contradicting the hypothesis that *A. hyacinthus*

on Palau recovered from a pulse recruitment of larvae from a single source. These data, however, do not exclude the possibility that Palau received a larval pulse from Yap and/or Ngulu, or from more distant populations such as Phoenix Island as demonstrated by Davies et al. (2015), which then mixed with larvae from surviving local populations.

The pairwise comparisons between islands and among sites show similar F'_{ST} values, highlighting the fact that there is as much differentiation among sites within a single island as among sites on different islands. Although all pairwise F'_{ST} values are significant ($p<0.01$) and there is clear population structure among all sites sampled in this study, it is important to note that F'_{ST} values are small enough to reflect some degree of gene flow through time. This result is more consistent with the prediction of high local retention of larvae in the simulations of Golbuu et al. (2012) than the hypothesized mass recruitment from Yap. Overall, these data support the idea that recovery of *A. hyacinthus* in Palau did not come exclusively from a single source. Instead, the lack of any evidence of a genetic bottleneck and the unique genetic diversity seen in Palau indicate that either mortality was less than 100% and that recovery came from a combination of areas, including sites within Palau. Further, these data indicate that the catastrophic mortality of *A. hyacinthus* reported by Bruno et al. (2001) was likely not as widespread as thought and that there were enough surviving colonies to reseed the barrier reef of Palau as well as maintain genetic diversity.

Structure of sites within Palau

Sites within Palau separated by as little as 5km show as much population genetic differentiation as sites between islands separated by as much as 452km. Both the pairwise and PCA analyses show that Palau's site S24 (West Palau) is as close to Yap sites S27, S29 and S30 or Ngulu site S28 as it is to Palau site S17 (East Palau). It is interesting to also note that site S17 shows the most differences with other Palau sites S20 and S24 and even greater differences with Yap and Ngulu sites. The eastern lagoon and reef of Palau has historically been impacted by development such as the construction of the airports and roads (Maragos and Cook 1995) and has been described as being impacted from sediment run-off (Golbuu 2011a; Golbuu et al. 2012). These anthropogenic impacts may have triggered similar population changes to those observed in terrestrial habitats when there is habitat degradation and fragmentation in terrestrial habitats which lead to an erosion of genetic variation and increased genetic divergence between

populations due to increased random genetic drift, elevated inbreeding, and reduced gene flow (Young et al. 1996).

Implications for Conservation

I show that populations of *A. hyacinthus* on Palau did not recover from a single pulse recruitment of larvae, and that mortality was likely less widespread than originally thought, with at least some pockets of surviving colonies within the Palauan archipelago that preserved unique genetic diversity there. Furthermore, there are significant differences among sites around Palau indicating that if there is exchange, it is insufficient to homogenize the populations, supporting the larval dispersal simulations of Golbuu et al. (2012) that there is a high level of self-recruitment among sites. In terms of conservation, these data support increasing the area of conservation by either increasing the number of MPAs or increasing the size of existing MPAs around Palau to protect a wide array of the genetic diversity.

Tables

Table 2.1. GPS coordinates, main island group and number of samples genotyped for each site. Sites marked with an asterisk are samples previously collected by (Davies et al. 2015).

Site	Island	GPS	Number
S17 East	Palau	7° 025’46.92 N, 134° 038' 31.272 E	48
S20 North	Palau	8° 000' 05.04 N, 134° 032' 09.960 E	48
S24 West	Palau	7° 031' 50.52 N, 134° 024' 03.960 E	48
S27 Goofnuw Channel *	Yap	9° 034’ 26.40 N, 138° 120’ 19.200 E	37
S29 West Outer Reef *	Yap	9° 033’ 47.30 N, 138° 050’ 71.500 E	48
S30 South Tip Reef *	Yap	9° 026’ 05.40 N, 138° 020’ 10.400 E	48
S28 Ngulu *	Ngulu Atoll	8° 180’ 12.00 N, 137° 290’ 18.700 E	43

Table 2.2. Number of alleles (in white) per locus and per site. Allelic richness (in grey) based on 24 colonies per locus and per site.

	Site 17		Site 20		Site 24		Site 27		Site 28		Site 29		Site 30		Total	
loc1	2	2.00	2	2.00	2	2.00	2	2.00	2	2.00	2	1.99	2	2.00	2	2.00
loc3	5	3.94	5	4.74	6	5.50	5	4.99	6	5.52	6	5.49	6	5.46	7	5.27
loc4	15	12.75	18	15.49	13	11.51	13	12.51	16	13.71	13	10.97	16	13.99	21	14.88
loc5	8	6.77	6	5.46	6	4.53	4	3.88	7	5.38	6	4.50	4	4.00	10	5.09
loc6	2	2.00	3	2.97	4	3.98	4	3.98	4	3.04	3	2.56	4	3.49	4	3.46
loc8	10	8.37	11	9.55	13	12.14	9	8.51	13	11.22	11	10.30	13	11.36	15	10.99
loc11	7	6.02	5	4.96	7	6.48	7	6.55	5	4.78	6	5.91	7	6.72	8	6.65
loc12	13	10.27	17	13.83	12	10.94	13	11.77	12	10.93	11	9.35	14	11.51	20	11.69
loc13	5	4.25	5	4.75	6	5.36	7	6.59	4	3.52	5	4.75	6	5.79	7	5.41
loc14	8	6.25a	7	6.20	11	9.39	6	5.48	8	7.26	10	8.40	5	4.54	13	7.69
loc16	1	1.00	2	1.98	6	4.92	5	4.78	2	2.00	5	4.38	6	4.99	7	4.15

Table 2.3. Comparison of the different datasets created by alternate strategies of filtering and scoring using AMOVA and indices of genetic diversity including number of individuals (*N*), effective number of individuals (N_E) expected (H_E) and observed (H_O) heterozygosities, corrected total heterozygosity (H'_T) global F_{ST} and F'_{ST} values, significance levels (*p*), and Nei's corrected fixation index G'_{ST} and significance level (*p*). Random_000 was generated by randomly selecting the second allele when there were more than two choices; topdepth was generated by selecting the two alleles with the highest number of representation among all colonies, random_min 10 was generated by randomly selecting the second allele on the dataset retaining only alleles that were present in more than 10 colonies, depth_min 10 was generated selecting the two alleles with the highest number of representation among all colonies on the dataset retaining only alleles that were present in more than 10 colonies. Two sets of files were generated: alleles were given a unique identification (_ID) or were identified by their length (_len).

	N	N_E	H_E	H_O	H'_T	F_{ST}	F'_{ST}	*p*	F_{IS}	*p*	G'_{ST}	*p*
N=321, Loci=11												
random000_ID	27.55	4.95	0.70	0.46	0.73	0.04	0.13	0.001	0.31	0.00	0.04	0.001
topdepth_ID	23.73	4.28	0.67	0.47	0.71	0.05	0.16	0.001	0.25	0.00	0.06	0.001
N=318, Loci=10												
random_min10_ID	12.80	4.20	0.66	0.44	0.70	0.04	0.13	0.001	0.30	0.00	0.05	0.001
depth_min10_ID	12.20	3.62	0.64	0.45	0.68	0.06	0.16	0.001	0.26	0.00	0.06	0.001
N=321, Loci=11												
random000_len	10.40	3.69	0.64	0.43	0.67	0.04	0.11	0.001	0.32	0.00	0.05	0.001
topdepth_len	9.90	3.28	0.62	0.44	0.65	0.06	0.14	0.001	0.27	0.00	0.06	0.001
N=318, Loci=10												
random_min10_len	9.83	3.93	0.67	0.43	0.65	0.04	0.11	0.001	0.31	0.00	0.05	0.001
depth_min10_len	8.00	3.02	0.59	0.42	0.63	0.06	0.14	0.001	0.27	0.00	0.06	0.001

Table 2.4. *F'ST* (above the diagonal) and significance values (below) of exact test of population differentiation (100,000 steps).

	Island						
	Palau			Yap			Ngulu
Site	*S17*	*S20*	*S24*	*S27*	*S29*	*S30*	*S28*
S17	--	0.05	0.12	0.23	0.16	0.20	0.17
S20	0.001	--	0.02	0.12	0.07	0.10	0.09
S24	0.001	0.002	--	0.08	0.04	0.07	0.09
S27	0.001	0.001	0.001	--	0.09	0.02	0.08
S29	0.001	0.001	0.001	0.001	--	0.03	0.10
S30	0.001	0.001	0.001	0.003	0.001	--	0.13
S28	0.001	0.001	0.001	0.001	0.001	0.001	--

Figures

Figure 2.1. Example of a colony of *Acropora hyacinthus* collected in Palau.

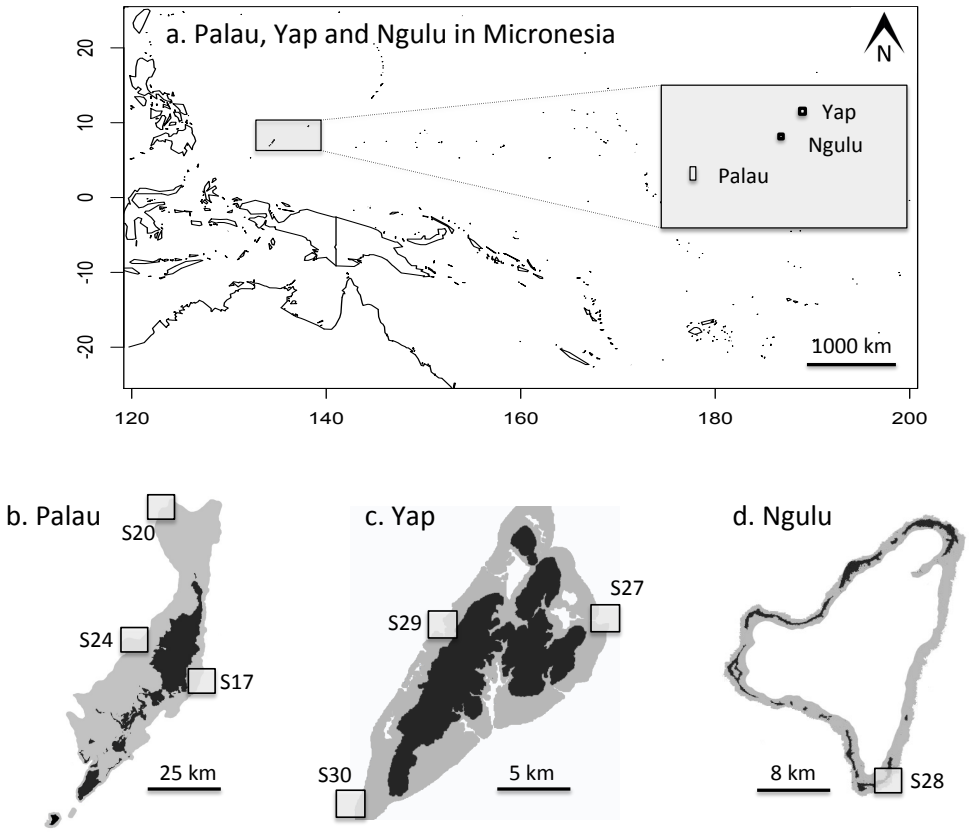

Figure 2.2. Maps of sampling locations: a. overview of location of Yap, Ngulu and Palau in Micronesia; b. sample sites in Palau; c. sample sites in Yap, d. sample sites in Ngulu.

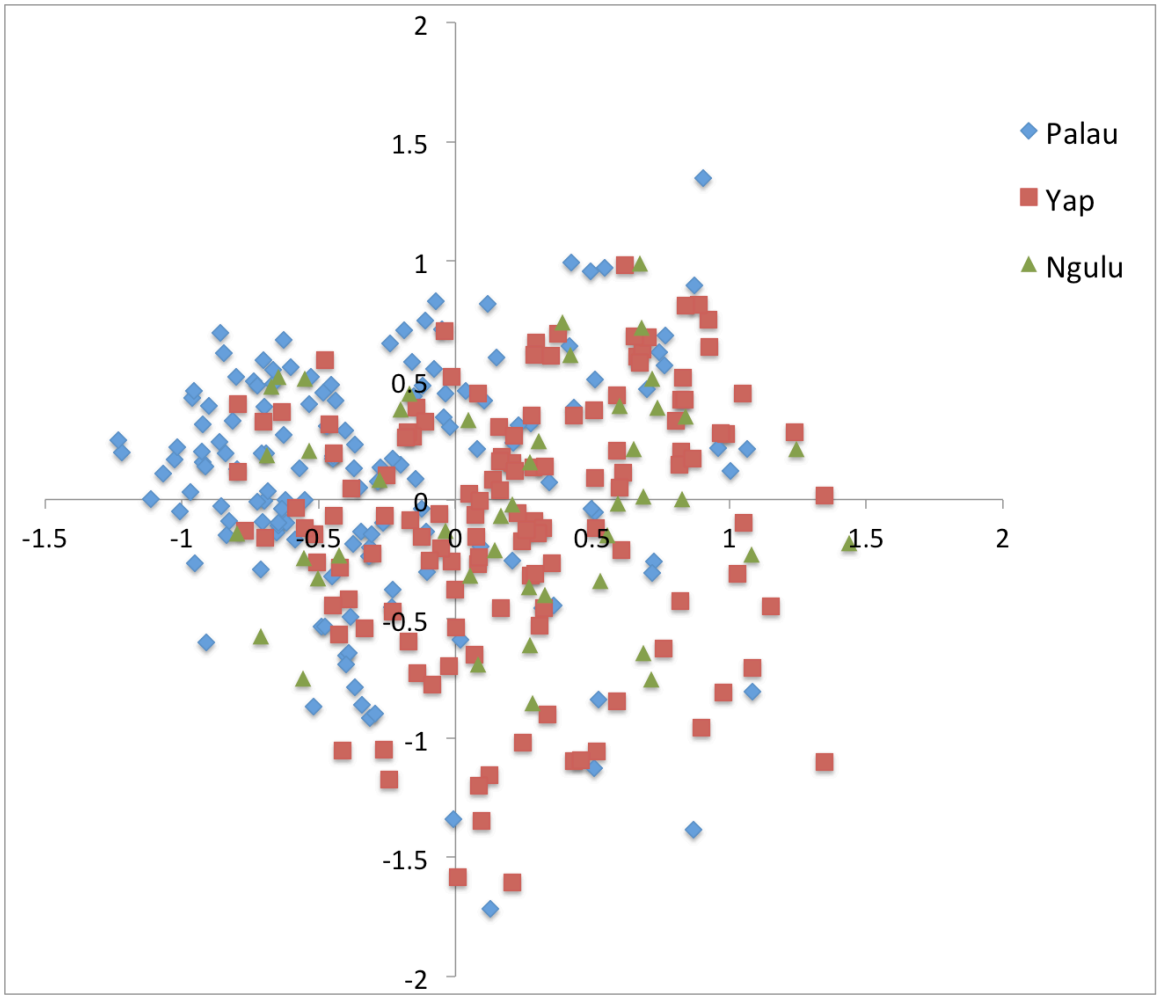

Figure 2.3. Principal component analysis on individuals for all sites. Results were color coded to show the island at which individuals were found. PCA axis 1 explains 7.2% of the variation and axis 2 explains an additional 5.7%.

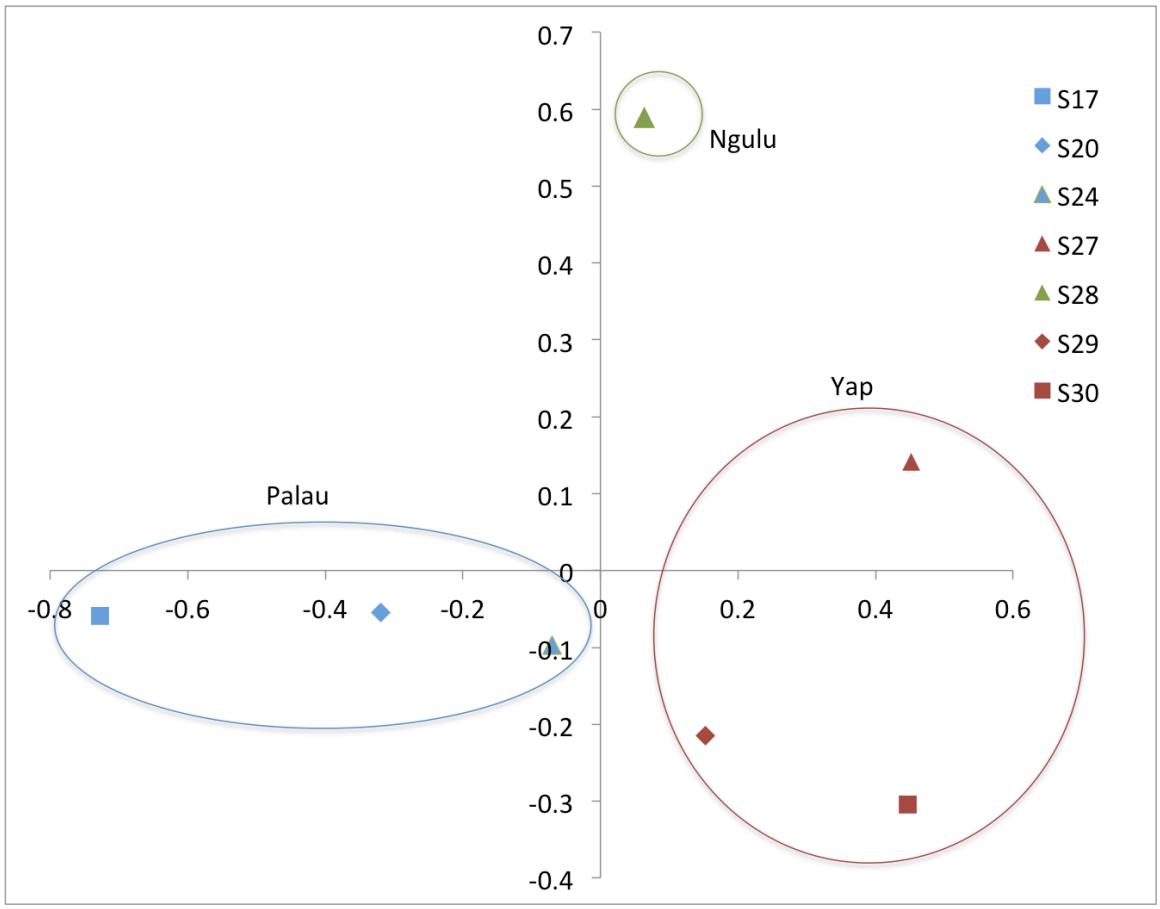

Figure 2.4. Principal component analysis on sites (populations). The first axis explains 38% of variation and second axis explains 24% of variation.

CHAPTER III. CONNECTING PALAU'S MARINE PROTECTED AREAS: A POPULATION GENETIC APPROACH TO CONSERVATION

Abstract

As bleaching events are becoming more frequent, they are impacting reefs around the world at different rates and formerly sporadic bleaching events are projected to become annual in Micronesia by 2040. To prepare for this threat, the government of Palau is reviewing its marine protected area network to increase the resilience of the reefs by integrating connectivity into the network design. To support their effort, I used high throughput microsatellite genotyping of the coral *Acropora hyacinthus* to characterize population genetic structure and dispersal patterns that led to the recovery of Palau's reefs from a 1998 bleaching event. I find no evidence of a founder effect or refugium where colonies may have survived to recolonize the reef. Instead, I found significant pairwise F'_{ST} values, indicating population structure and low connectivity among most of the 25 sites around Palau. I used kinship to measure genetic differences at the individual level among sites and find that differences were best explained by the degree of exposure to the ocean ($F_{1,20} = 3.015$, $Pr(>F) = 0.01$), but with little of the total variation explained. A permutation test of the pairwise kinship coefficients revealed that there was self-seeding within sites. Overall, the data point to the population of *A. hyacinthus* in Palau recovering from a handful of surviving colonies with population growth primarily from self-seeding and little exchange among sites. This finding has significant implications for the management strategies for the reefs of Palau and I recommend increasing the number and distribution of management areas around Palau to capture the genetic architecture and increase the chances of protecting potential refuges in the future.

Introduction

Coral reefs are declining due to a variety of anthropogenic impacts that are exacerbated by climate change (Hughes et al. 2003; Baker et al. 2008; McLeod et al. 2009; Burke et al. 2011). As atmospheric levels of CO_2 continue to rise, coral bleaching events are projected to increase in frequency and severity worldwide (Van Hooidonk, Maynard and Planes 2013); however, the rates of increases are predicted to vary between regions (van Hooidonk et al. 2013). In the best case greenhouse gas scenario (RPC6.0) where there is reduction and stabilization of CO_2 emission, bleaching events are predicted to occur annually by 2078 across reefs worldwide, with some areas, such as Micronesia, surpassing thermal bleaching thresholds annually as early as 2040 (van Hooidonk et al. 2013).

To increase the recovery of reefs affected by thermal stresses and bleaching, maintaining connectivity between populations has become central to conservation strategies (Jones et al. 2007, 2009; Almany et al. 2009; McLeod et al. 2009). Networks of marine protected areas (MPAs) may promote recovery from disturbance by allowing reefs to be mutually replenishing through coral recruitment (Hughes et al. 2003; Salm et al. 2006; IUCN 2008; McLeod et al. 2009). Even the best-studied and best-funded MPA networks such as the network on the Great Barrier Reef, however, did not consider connectivity between their no-take reserves when rezoned because they lacked the information (Fernandes et al. 2005). The absence of connectivity data is a challenge for most MPA networks around the world due to the high cost of producing this information for managers (Almany et al. 2009; Lagabrielle et al. 2014; Magris et al. 2014).

Connectivity of marine organisms is particularly difficult to measure due to the small size and high dispersal potential of pelagic larvae (reviewed in (Levin 2006; Cowen and Sponaugle 2009). Two main approaches are generally used to determine population connectivity: (i) computer modeling to simulate the dispersal of particles forced with physical data (e.g., wind and tides), and (ii) molecular data (i.e., DNA) to study gene flow between populations (Hellberg 2007; Cowen and Sponaugle 2009). Seascape genetics brings together both approaches to test for environmental drivers of spatial genetic

structure (Selkoe et al. 2008, 2016; White et al. 2010; Liggins et al. 2013; Riginos and Liggins 2013), allowing cross-validation between oceanographic models and genetic data (Baums et al. 2006b; Pringle and Wares 2007; White et al. 2010; Foster et al. 2012) and improved application to conservation purposes (Selkoe et al. 2015). In this study, I use genetic techniques to test whether the connectivity predicted by an oceanographic model of Palau developed by Golbuu et al. (2012) coincides with genetic differences on Palau's reefs.

Palau is the western-most island in Micronesia, which has been identified as one of the areas that will be exposed to bleaching conditions annually by 2040 (van Hooidonk et al. 2013). Having experienced heavy mortality on the barrier reef in a 1998 bleaching event, the government of Palau is responding to future thermal stresses by reviewing the design of the network of MPAs to increase its resilience. To inform the placement of new MPAs, Golbuu et al. (2012) used a two-part oceanographic model predicting long-distance dispersal between Yap and Palau incorporating the currents and wind data as well as a "sticky water" model developed specifically to predict larval retention around reefs locally (Wolanski 1994; Wolanski and Spagnol 2000; Andutta et al. 2012). The long-distance model describes the potential dispersal of coral larvae from Yap to Palau. The localized model describes low self-seeding for most of the barrier reef and an export of larvae from the southern barrier reefs to the northern barrier reefs. Golbuu et al. (2012) tested their model against coral cover and density of juvenile *Acropora* colonies. They calculated self-seeding and density of larval recruits as the sum of self-seeding and larvae from other sites. The authors found that areas of high coral cover corresponded to areas of predicted high self-seeding and areas of high density of juvenile *Acropora* colonies corresponded to areas of high density of larval recruits. Although there is a correlation between coral cover and expected self-seeding, for example, there is no evidence that the corals colonies are more related in areas of high coral cover than in with lower cover.

Cros et al. (2016) showed that it was unlikely that reefs in Palau recovered from a pulse event from Yap and that genetic distances between Yap and Palau are similar to genetic distances between sites around Palau and they hypothesized that recovery may have

occurred from surviving colonies in Palau. To better understand how Palauan reefs recovered from the 1998 bleaching event and to compare the oceanographic dispersal model (Golbuu et al. 2012) to genetic structure, I assess the genetic structure and genetic diversity of populations of *Acropora hyacinthus* around the reefs of Palau. The objective is to understand the patterns of dispersal that have led to recovery, detect evidence of self-seeding and to provide recommendations to increase resilience of the reefs in Palau.

Methods

Study species

Acropora hyacinthus is a table coral found on shallow barrier reefs between three and 10m throughout the Indo-Pacific. Although it is one of the dominant coral species growing on Palau's barrier reef (Golbuu et al. 2007; Victor et al. 2009), *A. hyacinthus* is rare or absent on the patch reefs, fringing reef and lagoon of Palau (Bruno et al. 2001). In 1998, *A. hyacinthus* suffered heavy mortality from a bleaching event in Palau, almost disappearing from the reef (Bruno et al. 2001), but by 2005 it was once more dominant on the reef slopes (Golbuu et al. 2007). Two hypotheses were put forward for this rapid recovery. The first is that Palau received an important recruitment pulse of larvae from Yap (Golbuu et al. 2012) , however, Cros et al. (2016) demonstrate that there is no genetic evidence to support mass recruitment from Yap to Palau. The second hypothesis is that there were more surviving colonies than originally described by Bruno et al. (2001) and reefs not surveyed by Bruno et al. (2001) may have been refugia with surviving colonies acting as a source of recruits to recolonize the reef (Golbuu et al. 2007; Victor et al. 2009 and Cros et al. 2016).

A. hyacinthus is a hermaphroditic broadcast spawner that releases egg and sperm bundles during mass spawning events (Ayre and Hughes 2000). Although *A. hyacinthus* can also reproduce asexually, previous studies have found very few clones in the field (Ayre and Hughes 2000; Márquez et al. 2002). *A. hyacinthus* reaches maturity between three to five years (Wallace 1985). After successful fertilization, under laboratory conditions planktotrophic *A. hyacinthus* larvae (Toh et al. 2013) can remain in the water column for up to 90 days before settling (Márquez et al. 2002). For populations of *Acropora* on the

Great Barrier Reef, however, Ayre and Hughes (2000) find that there is genetic structure between reefs separated by a few kilometers, and Hughes et al. (2000) find that reefs where colonies have low reproductive outputs also have low recruitment levels. Therefore, despite a potential larval duration of 90 days (Márquez et al. 2002), genetic data (Ayre and Hughes 2000; Hughes et al. 2000) indicate that dispersal on reefs may be highly localized.

Sampling locations and methodology

In February and May 2012, 25 sites on the outer barrier reef of Palau were sampled at a shallow depth (<10m) using SCUBA (Fig. 3.1, Table 3.1). The outer barrier reef was divided into four quadrants with an east-west division along the length of the atoll and a north-south division separating the northern lagoon and surrounding reefs from the southern lagoon and the reefs adjacent to the two main islands of Babeldaob and Koror. Sites were selected within each area to represent a range of habitat and wave exposure characterized by the West Pacific Monsoon winds and northeasterly trade winds. During winter (December to March) trade winds are blowing and forming large waves from the northeast. In the summer, the monsoon winds are dominant and smaller waves are coming from the west and northwest Palau (Australian Bureau of Meteorology and CSIRO, 2014). Sites within each of the four zones, northeast, northwest, southeast and southwest are more or less exposed at a the similar angle to these waves. Additionally, sites both within and outside MPAs were included to represent management categories found on the barrier reef. A total of 1200 x $1cm^3$ colony tips were collected by sampling haphazardly 48 colonies of *A. hyacinthus* in 4 x $200m^2$ belt transects at each of these 25 sites. Branch tips were preserved in salt-saturated DMSO at room temperature (Gaither et al. 2011).

DNA extraction and sequencing

A detailed description of DNA extraction and sequencing is described in Cros et al. (2016). Briefly, genomic DNA was isolated from each branch tip and eighteen microsatellite loci (Appendix 6) were amplified using a forward primer with a short tag to create 48 unique colony IDs for each microsatellite locus (Appendix 2). The barcoded amplicons were pooled by site. An Illumina adaptor (Illumina Inc., Hayward, CA, USA)

was ligated to generate a library with the following unique structure: siteID-colonyID-FWDprimer-flankingregion-tandemrepeats-flankingregion-RVSprimer. Each library was sequenced on an Illumina MiSeq.

Data processing

Raw sequences were processed following the bioinformatics pipeline in Cros et al. (2016). In brief, the sequences were demultiplexed by site, merged, separated according to primer and colony and trimmed for low quality sequences. They were then collapsed into unique sequences and counted. In order to eliminate PCR and sequencing artifacts, a set of filters was developed in PYTHON (https://github.com/annickcros/Ahyacinthus-filters.git). Flanking regions were filtered from simple tandem repeats (STR) using EMBOSS: ETANDEM (Rice et al. 2000). Genotypes were created based on the STR. Data was transformed to GENODIVE v. 2.0b27 (Meirmans 2009) file format using formatting as described in Cros et al. (2016). The final dataset analysis was carried out on 11 loci (Table 3.2) after eliminating loci with over 15% missing data. The final number of colonies analyzed for each locus varies between 37 to 48 per site.

Analyses

Population differentiation

I first tested for clones using GENODIVE v. 2.0b27. Finding none, I conducted the analysis on single ramets. To characterize the genetic structure of each of the 25 sites, I assumed that each site was a single population and calculated in GENODIVE the number of alleles, the effective number of alleles, and indices of genetic diversity, as well as observed (H_O) and expected (H_E) heterozygosities, and inbreeding coefficient G_{IS} (Table 3.3) at each site. To test for overall population structure among the 25 sampled sites, I calculated global F_{ST}, F'_{ST} and F_{IS} and corresponding p values with an AMOVA in GENODIVE (Table 3.4). Indices of global genetic diversity, including observed (H_O), expected (H_E) and corrected heterozygosities (H'_T), inbreeding coefficient (G_{IS}) and Nei's fixation index G_{ST}, were also calculated (Table 3.4).

To test the hypothesis that *A. hyacinthus* recovered from a few individuals surviving the 1998 bleaching event, I looked for evidence of recent bottlenecks or founder effects using

BOTTLENECK 1.2.02 (Piry et al. 1999). I used the graphical test from Luikart et al. (1998) based on a mode shift away from an L-shaped distribution of allelic frequencies. I used the Wilcoxon signed-rank test (10,000 iterations) using both a two-phase (TPM incorporated 70% stepwise and 30% multistep mutations) and an infinite allele (IAM) mutational model due to the relatively small number of microsatellite loci scored in our dataset.

To test for connectivity between sites and patterns of genetic structure, I used pairwise differentiation tests between sites in GENODIVE and reported F'_{ST} and p values in Table 3.5 Jost's D values were also estimated (Appendix 7). I also looked for patterns of genetic structure by performing a Principal Component Analysis (PCA) in GENODIVE (Meirmans 2009) on sites using a covariance matrix of allele frequencies with 10,000 permutations. The graphs were plotted in EXCEL (Microsoft, Redmond, Washington, 2010, Fig. 3.2).

To examine spatial genetic structure of *A. hyacinthus*, I carried out a Bayesian clustering algorithm implemented in STRUCTURE ver. 2.3.4 (Pritchard et al. 2000). I used a no-admixture model with location as a prior and a burn-in of 10,000 chains followed by 10,000 MCMC replications as suggested by Pritchard (2010) and Benestan et al. (2016). Twenty independent runs were carried out for each number of clusters (K) from 1 to 25. The most likely value of K was evaluated using the method of Evanno et al. (2005) in CLUMPAK (Kopelman et al. 2015).

Patterns of connectivity

I created a pairwise matrix of geographic distances by measuring the shortest distance between each pair of sites following the contour of the barrier reef using ESRI ARCGIS v.10.2.2. (Appendix 8). I tested for isolation-by-distance with a Mantel test (Mantel 1967) in GENODIVE comparing a matrix of transformed pairwise F'_{ST} values (F'_{ST}/ F'_{ST}-1) to a matrix of log-transformed geographic distances with 20,000 permutations. I also used the Isolation By Distance Web Service (IBDWS) (http://ibdws.sdsu.edu/~ibdws/) to test for a relationship between F'_{ST} and distance, with and without log-transformation.

To compare the dispersal patterns predicted by Golbuu et al. (2012) with genetic differentiation, I overlaid the sampling sites from this study with the map of self-seeding rates (i.e., the percentage of particles remaining in the site after 120 hours) and the map of total seeding rate (i.e., the sum of particles retained at a given site plus the imports from other release sites after 120 hours) from Golbuu et al. (2012). Sites were grouped according to the level of self-seeding and total seeding on the barrier reef. For self-seeding, I used the following groups (Table 3.1): High (60-65%), 1 site; Medium (16-25%), 3 sites; Low (0-15%), 19 sites. For total seeding, I used the following groups: High (61-90%), 6 sites; Medium high (41-60%), 11 sites; Medium low (21-40%), 3 sites; Low (0-20%), 4 sites. I performed a hierarchical AMOVA in GENODIVE using these groupings. I also performed a "group comparison test" in GENODIVE that tests whether groups of populations differ in their values of certain summary statistics. The group comparison test calculates summary statistics for each group and then uses a permutation test to test for differences between the groups. The OSx-statistic was used (Goudet 1995), which is the sum of the squared differences in the test statistic over all pairwise combinations of groups. Permutations take place by randomizing the populations over the groups (Meirmans 2009). I compared H_O and H_E and indices of genetic diversity G_{IS} (inbreeding coefficient) and G_{ST} (fixation index) to compare the self-seeding and total seeding groups (Table 3.6).

I repeated the same methods as described above, performing an hierarchical AMOVA and a group comparison in GENODIVE to test for a genetic division between the sites grouped by western and eastern reefs, by southern and northern reefs, and by zones characterizing exposure to wind and waves (exposure zones): northeast, northwest, southeast and southwest reefs (Fig. 3.1, Tables 3.1 and 3.7).

I tested for self-seeding by comparing (i) pairwise relatedness of individuals within sites and between sites using ML-RELATE (Kalinowski et al. 2006) and (ii) pairwise kinship coefficients of individuals within sites and between sites using GENODIVE. For both pairwise relatedness and pairwise kinship, I compared distributions of within-site and between-site pairwise comparisons. To test for differences in mean pairwise kinship

between within-site and between-site pairs (Fig. 3.3a), I resampled without replacement (resample size = 27,646, number of resamples = 1,000) from the distribution of all pairs and calculated the mean difference between within-site pairs and between-site pairs (Fig. 3.3b). Sites with high within-site pairwise kinship coefficients indicate sites with high self-seeding and sites with high between-site pairwise kinship coefficients indicate sites with high total seeding. I ranked the mean pairwise kinship coefficients within sites and among sites and compared them with the predicted self-seeding and total seeding groups as defined by Golbuu et al. (2012) (Appendix 9).

I used a canonical analysis of principal coordinates (CAP) to see which of the following predictor variables explained the most genetic variation: distance (Appendix 8), differences between northwest exposure, eastwest exposure and their interaction. I carried out an ANOVA to test for the importance of the predictors. All of these analyses were carried out in R v. 3.2.3 (R Core Team 2015) (package vegan 2.3-5).

Results

Population differentiation

I genotyped between 44 to 48 colonies at each site (Table 3.1) for each of the 11 microsatellite loci. At each site, the effective number of alleles per locus varied between 3 and 4.5 (Table 3.3). Observed heterozygosity (H_O) ranges from 0.22 to 0.53 and expected heterozygosity (H_E) from 0.51 to 0.66 and all but two were out of Hardy Weinberg Equilibrium (HWE, Table 3.3).

Observed heterozygosity averaged over all sites and loci (H_O = 0.39, ±0.06 SD) shows a lower value than the expected heterozygosity averaged over all sites and loci (H_E = 0.61, ±0.079 SD) and a higher inbreeding coefficient than expected (G_{IS}=0.37, p<0.01). Both the fixation index F_{ST} and the standardized G_{ST} (Nei) are low but significant (p<0.01), indicating fine-scale population structure among the 25 sites around Palau (Table 3.4). Likewise, pairwise F'_{ST} comparisons indicate that most of the 25 sites around Palau are significantly different from each other (Table 3.5). Pairs of sites that are not significantly different are not organized in any obvious geographical patterns.

The base-shift graphical test for bottleneck showed normal L-shaped distributions for all 25 sites, indicating no evidence of a bottleneck for *A. hyacinthus* around Palau.

The results from STRUCTURE and CLUMPAK indicate the most likely number of clusters based on the method of Evanno et al. (2005) is 4. Summary graphs, however, do not show a clear separation between three populations (data not shown). Similarly, the PCA does not highlight any obvious spatial patterns or geographic division among sites, with all sites distributed fairly evenly along the first and second axis (Fig. 3.2).

Patterns of connectivity

I found no evidence of isolation-by-distance. There is no significant relationship between either the untransformed (Spearman's r = 0.024, p = 0.270) or log-log transformed (Spearman's r = -0.0038, p = 0.469) geographic and genetic distances.

I overlaid the sampling sites from this study with the map of self-seeding rates and the map of total seeding rate from Golbuu et al. (2012). I tested to see if there was significant between sites grouped according to the self-seeding and total seeding classes defined in Golbuu et al. (2012). The hierarchical AMOVA in GENODIVE did not indicate significant genetic differences (F'_{ST}) between sites grouped by self-seeding classes or the total seeding classes and I find $F_{CT\,(SELF)} = 0.001$ ($p = 0.37$) and $F_{CT\,(TOTAL)} = 0.003$ ($p = 0.12$). Similarly, the OSx-statistic of the group comparison test in GENODIVE did not reveal any significant differences in either the value of observed or expected heterozygosities, inbreeding coefficient or fixation index *GST* between sites with high, medium, or low self-seeding rates or between sites with low, med-low, med-high, or high total seeding rates (Table 3.6).

The AMOVA hierarchical analysis for sites grouped into north and south and east and west did not show any significance differences between groups, however, it did showed a significant difference for sites grouped by exposure zones ($F_{CT} = 0.006$ and $p<0.05$). In addition, the group comparison for the exposure zones showed significant differences in observed and expected heterozygosities and inbreeding coefficients (Table 3.7) with the

sites grouped in the northwest exposure zone showing the lowest inbreeding coefficients and driving the observed difference.

The overlaid histograms of the distribution of the frequency of the pairwise kinship coefficient within and between sites indicate that there are higher kinship coefficients within sites than between sites (Fig. 3.3a). I plotted the results of the permutation analysis, testing the significance of the difference between the mean of pairwise kinship coefficients within and between sites (Fig. 3.3b), and noted that the distribution of the mean differences between permuted within and between site pairs are squarely distributed around zero. The value of the mean pairwise kinship coefficient within sites is 0.0286 (95% CI = -0.0013 - 0.0011), between sites is -0.0012 (95%CI= -0.0001- -0.00002) and the difference in means is 0.0298 (95%CI = -0.0012 - 0.0012). The within and between site distribution fall outside of the 95% percentile indicating that the mean of pairwise kinship coefficients for within and between sites are significantly different. Analyses of relatedness gave similar results.

The four sites with the highest within-site mean kinship coefficients were S17 and S23 in the southeast and S2 and S3 in the southwest (Appendix 9). This does not correspond to the class given by Golbuu et al. (2012) where S17, S23 and S2 are classed as "low" for self-seeding. The four pairs of sites with highest between-site mean kinship coefficients were S23×S25, S3×S4, S22×S23 and S4×S5 (Appendix 9). This matches predictions by Golbuu et al. (2012) a little better where S4, S5 are classed as "high" for total seeding and S22 as "medium-high". Overall kinship coefficients and self-seeding and total seeding classes do not match.

Variation in genetic differentiation between sites was best explained by exposure zones, which was significant in the ANOVA ($F_{1,20}$= 3.015, Pr(>F) = 0.01) and explained 31% of the of the total variation.

Discussion

Understanding the processes by which *A. hyacinthus* has recovered from the devastation of the 1998 bleaching event is critical for the future management of these reefs, to better

decide on the placement of new MPAs and to help protect areas that may act as refugia. I previously documented that the hypothesis that recovery came from a massive influx of recruits from the island of Yap (Golbuu et al. 2012) was inconsistent with the genetic patterns among these sites (Cros et al. 2016). Here, I tested the hypothesis that recovery was generated from a few surviving colonies and further compared the modeled larval dispersal from Golbuu et al. (2012) to determine how well it predicted the observed genetic diversity among sites in Palau. I present a series of hypothesis on the processes that resulted in the genetic patterns I observe and propose different scenarios for the recovery of the reef.

Founder Effect

If there had been a recolonization of the population of *A. hyacinthus* on the barrier reef from only a few colonies that had survived the 1998 bleaching event, there would be signs of founder effects in the new populations. Yet the bottleneck analysis shows no disproportionate distribution of allelic frequencies. One possible explanation is that there were more surviving colonies than expected, eliminating any evidence of a founder effect. Gaither et al. (2010) report that in a review by Dlugosch and Parker (2008), among 11 cases of intentional introduction where the number of individuals was known confidently and derived from a single source population, loss of genetic diversity was detected in all but one case involving the introduction of less than 250 individuals. In their own observation of the introduction of groupers in Hawaii, Gaither et al. (2010) find signs of bottleneck for introductions of less than 750 of adults. If this result is consistent, it would indicate that at least ~250-750 colonies of *A. hyacinthus* survived on over 400km^2 of barrier reef around Palau hiding effects on any bottleneck.

Self-seeding and differential survival

In addition to the lack a bottleneck effect, I find that most of the 25 sites around the barrier reef of Palau display genetic differences with significant F'_{ST} for the majority of the pairwise comparisons (Table 3.5). At the same time, the distribution of kinship coefficients within and between sites shows evidence of self-seeding (Fig. 3.3a and b). A possible hypothesis to explain the processes that drives the genetic diversity of *A. hyacinthus* around Palau's barrier reef is that enough *A. hyacinthus* colonies survived the

1998 bleaching to recolonize the reef and that their larvae mostly recruited close to parental colonies creating genetic structure between sites. Another possible explanation is that there was differential survival of recruits such that even with some gene flow, individuals at a site grow to be differentiated through time (Toonen and Grosberg 2011; Gorospe and Karl 2015). An alternative explanation is that I sampled cryptic species (Ladner and Palumbi 2012) but I see no evidence for linkage groups in either the STRUCTURE analysis or the PCA.

Reef patchiness and clumped recruitment

Pinsky et al. (2012) modeled the effect of habitat patchiness on population openness and tested it on different reefs. They found that in many cases, habitats were sufficiently patchy at scales of tens of kilometers to create largely closed populations, in particular for species with low dispersal abilities. Habitat patchiness can be the result of disturbance, in particular coral mortality due to bleaching (Andréfouët et al. 2002; Hughes et al. 2003). In the case of Palau, it is likely that a handful of *A. hyacinthus* survived in patches and although *A. hyacinthus* larvae have the potential for long distance dispersal, there is increasing evidence that, like other marine organisms, the realized dispersal is considerably more restricted (Hughes et al. 2000; Levin 2006; Shanks 2009; D'Aloia et al. 2013). Under this scenario, the expectation of partially closed populations on the reef of Palau matches the results of F'_{ST} and kinship coefficient and would explain the lack of a strong genetic bottleneck. The existing patches mostly grow through self-seeding with few larvae being exported and starting new patches that are not completely closed and therefore mixing over several generations. Alternatively, there are larval exchanges over longer distances but sibling larvae are all transported in a batch and settle together, regardless of where they come from, creating genetic patchiness (Selkoe et al. 2006; Bernardi et al. 2012; Iacchei et al. 2013).

Small-scale selection

Self-recruitment at each site may also be influenced by small scale selection driven by environmental factors (Selkoe et al. 2010; Sanford and Kelly 2011; Toonen and Grosberg 2011). In a recent study, (Gorospe and Karl 2015) show that *Pocillopora damicornis* displays a continuous genetic gradient along a depth gradient of a few meters suggesting

that recruiting larvae may be selecting for a similar depths as their colony of origin and structuring intra-reef genetic diversity. Other examples in the literature include the work from Barshis et al. (2010) who found a significant differentiation between populations of *Porites lobata* sampled from an exposed fore reef and protected back reef and attributed this small-scale population subdivision to habitat characteristics. Similarly, Sherman et al. (2006) showed genetic variation between lagoon and reef slope populations of *Pocillopora damicornis*, which they attributed to wave exposure and consequent habitat heterogeneity. In Palau, I find the four different exposure zones best predicted the genetic difference among distance, north-south and east-west division. The effect of zones could be due to a nonlinear effect of distance on genetic variation. Each zone represents a different exposure to waves and could indicate selection on the genetic makeup of the populations of *A. hyacinthus*. There is, however, a lot of unexplained variance remaining from unexplored variables (Selkoe et al. 2016).

Oceanographic influence

I tested these genetic patterns against the predictions of the oceanographic larval dispersal model developed by Golbuu et al. (2012). The model predicts that the southern reefs contribute the most to total seeding, in particular of the northern reef which exports most of its larvae early on to open ocean. In contrast to the predictions of their model, I see no evidence of increased relatedness in areas defined as high self-seeding (Table 3.6). I see an increase in heterozygosity and decrease in inbreeding coefficients, however, for sites grouped in the northwest exposure zone (Table 3.7). In support of their model, I find evidence of self-seeding in sites in the southern reef. Higher exchanges between sites is restricted to the northern reefs and may indicate that there is more dispersal between sites in the northern lagoon, but not enough to remove all genetic structure.

Management Implications

Regardless of the underlying mechanism, here I show significant population structure of *A. hyacinthus* at a scale of tens of kilometers around Palau. This fine-scale population structure reflects diversity in genetic material, which often introduces variability in population resilience to stresses, including coral bleaching (Hughes et al. 2003; Palumbi et al. 2014), increasing the chance of some colonies surviving. These resistant colonies

can play a key role in supporting the recovery of more resistant coral reefs and in maintaining genetic diversity as demonstrated in the history of coral recovery in Kāne‘ohe, Hawai‘i (Bahr et al. 2015). Our study supports previous observations by Victor et al. (2009) and Golbuu et al. (2007) who found no relationship between recruitment densities and coral cover in 2004 and argued that recovery was most likely due to some surviving colonies. The catastrophic mortality reported by Bruno et al. (2001) apparently did not result in 100% mortality of *A. hyacinthus* as suggested, and our data indicate that there were enough surviving colonies to hide any effect of a genetic bottleneck.

The reason why these colonies survived is important for management implications. There are two likely scenarios. Either the colonies that survived were in an area that provided refugia to thermal stressors, such as a locally cooling current or on a slope that provided shading. In this case the surviving colonies are not particularly more resistant than the colonies that died and the growth of the reef from self-seeding will not result in a more resilient reef. The second scenario is that the colonies that survived were more resistant to thermal stresses or experienced previously exposed to non-lethal thermal stresses anomaly frequencies leading to higher adaptive capacity in which case self-seeding will result in a more resilient reef (Thompson and van Woesik 2009; Mumby et al. 2011; Oliver and Palumbi 2011; Bahr et al. 2015).

In the first case scenario of refugia, conservation agencies, such as The Nature Conservancy, recommend that resilient MPA networks should be designed to capture and protect as many of these refugia as possible (Salm et al. 2006; McLeod et al. 2009; Chollett and Mumby 2013). In the case of Palau, this recommendation would result in increasing the number and increasing the spacing of MPAs along the barrier reef at each of the wave exposure gradients. In the second case scenario, where surviving colonies are resistant to thermal stresses, conservation agencies focus on these communities to protect them from other threats and to adjacent habitats to enable dispersal and recruitment as much as possible (Selig et al. 2012). If only a few surviving colonies are broadly distributed, however, they may well fall outside of MPAs, and their protected would

require to increase the number and size of MPAs. Our data support an alternative strategy to increase the likelihood of survival in the case of repeated thermal stress events. To allow for recovery from these rare surviving colonies, it is important to have the best habitat possible for recruitment and growth. One solution to enable good habitat outside of MPAs is to manage the reef outside of the existing no-take areas and reserves to maintain key ecological functions of the reef and allow for recruitment (Steneck et al. 2009).

For coral recruitment, the key is to maintain a substrate conducive to larvae settling and surviving. Some essential elements to maintain recruitment include controlling for algae, sedimentation and water quality (Kuffner et al. 2006; Mumby et al. 2007; Burke et al. 2011). Palau already has restrictions on fishing and the coral reef ecosystem is functional and resilient (Victor et al. 2009). Thus, although the protection of herbivores can be a good strategy in some areas (Lewis 1986; Hughes et al. 2007; McClanahan et al. 2011), it may not be the case for Palau, which does not have any record of coral recruitment being hampered by algae with the exception of an algae bloom after a typhoon in 2013 which resulted in a temporary coral recruitment failure at a local level (Doropoulos et al. 2014). In contrast, the negative impacts of sedimentation and terrestrial runoff on coral reefs is well known (Hughes 1994; Bellwood et al. 2004) and have been documented as major anthropogenic impacts in Palau as well (Golbuu et al. 2007, 2011; Golbuu 2011b). Controlling for these land-based stressors may be a good strategy to focus on if resources are limited, particularly if the colonies surviving a mass bleaching are rare and sparsely spaced, any additional loss could substantially delay local recovery.

The genetic data presented here contradict the earlier conclusion by Golbuu et al. (2012) that Palau recovered from a pulse recruitment event from Yap. Investing in a region-wide marine protected area network to connect Yap to Palau would not bring the level of resilience to the reefs of Palau that the Palauan government and The Nature Conservancy are aiming for. This study highlights that in the case of Palau, focusing management actions locally to increase the chances of capturing the right environmental factors to act

as refugia and increasing the chances of individual coral colonies surviving catastrophic events is most critical to the recovery and survival of their reefs

Tables

Table 3.1. Reef site collections. Site number, seeding category, exposure, date of collection, GPS coordinates and number of samples collected. Self-seeding categories are defined based on levels of self-seeding in the Golbuu et al. (2012) model as follow: High (60-65%); Medium (16-25%); Low (0-15%). For total seeding, I used the following groups: High (61-90%); Medium high (41-60%); Medium low (21-40%); Low (0-20%).

	Seeding						
Site	**Self**	**Total**	**Exposure**	**Date of collection**	**Longitude (N)**	**Latitude (W)**	**# ind**
S1	Low	Med-High	SE	14.02.12	7.2874	134.50295	48
S2	Low	High	SW	18.02.12	7.5610	134.46864	47
S3	Medium	High	SW	16.02.12	7.4183	134.34557	48
S4	Medium	High	SW	17.02.12	7.3070	134.23141	47
S5	N/A	N/A	SW	20.02.12	7.0111	134.21833	44
S6	N/A	N/A	NE	11.03.12	8.0421	134.68630	46
S7	Low	Med-High	SW	22.02.12	7.2523	134.22093	45
S8	Low	Med-High	SE	21.05.12	7.2619	134.54426	48
S9	Low	Med-High	SE	21.05.12	7.3623	134.61971	48
S10	Low	High	SE	22.05.12	7.1113	134.36692	48
S11	Low	Med-Low	NE	23.05.12	7.9902	134.65965	48
S12	Low	Low	NE	23.05.12	7.9886	134.70319	48
S13	Low	Low	NE	24.05.12	7.8788	134.68135	48
S14	Low	Low	NE	25.05.12	7.8150	134.66043	47
S15	High	Med-High	NE	26.05.10	7.6678	134.64970	48
S16	Low	Med-High	NE	26.05.11	7.5860	134.64929	48
S17	Low	Med-High	SE	26.05.12	7.4297	134.64202	48
S18	Low	Med-High	SW	28.05.12	7.0796	134.26157	48
S19	Low	High	NW	29.05.12	7.7224	134.56752	48
S20	Low	Med-High	NW	31.05.12	8.0014	134.53610	48
S21	Low	Med-High	SE	01.06.12	7.0556	134.31810	48
S22	Low	Med-High	NW	02.06.12	7.8602	134.50802	48
S23	Low	Med-Low	SE	04.06.12	7.1633	134.41277	48
S24	Low	High	SW	05.06.12	7.5307	134.40110	48
S25	Low	Med-Low	NW	06.06.12	7.8018	134.50800	48

Table 3.2. Number of alleles (*A*), observed heterozygosity (H_O) with standard deviation (SD) and range of length (*nt*) of microsatellites loci.

Locus	***A***	***H_O* (SD)**	***nt***
Locus 1	2	0.34±0.10	28-36
Locus 3	6	0.69±0.07	21-36
Locus 4	20	0.91±0.02	15-75
Locus 5	11	0.55±0.05	57-90
Locus 6	4	0.50±0.11	36-45
Locus 8	14	0.83±0.03	39-108
Locus 11	8	0.66±0.06	12-44
Locus 12	17	0.83±0.05	36-93
Locus 13	8	0.72±0.08	54-81
Locus 14	12	0.68±0.07	20-84
Locus 16	7	0.32±0.24	32-56

Table 3.3. Indices of genetic diversity for each of the 25 sites around Palau, including number of alleles (N), effective number of alleles (N_E), expected (H_E) and observed (H_O) heterozygosities, inbreeding coefficient (G_{IS}) and significance levels of inbreeding coefficients (p).

Site	N	N_E	H_O	H_E	G_{IS}	p-**value**
S1	8.36	4.35	0.27	0.66	0.60	<0.01
S2	7.27	3.99	0.31	0.65	0.53	<0.01
S3	7.46	4.03	0.31	0.66	0.52	<0.01
S4	7.64	4.15	0.35	0.66	0.48	<0.01
S5	6.36	3.71	0.27	0.66	0.59	<0.01
S6	7.64	4.00	0.28	0.66	0.57	<0.01
S7	6.64	3.70	0.22	0.65	0.67	<0.01
S8	7.55	4.35	0.37	0.65	0.43	<0.01
S9	7.64	3.92	0.40	0.63	0.37	<0.01
S10	6.64	3.56	0.37	0.62	0.42	<0.01
S11	8.55	3.87	0.40	0.61	0.35	<0.01
S12	7.55	3.39	0.45	0.58	0.22	<0.01
S13	7.46	3.55	0.49	0.60	0.19	<0.01
S14	8.09	4.42	0.37	0.66	0.44	<0.01
S15	7.46	3.98	0.33	0.64	0.48	<0.01
S16	8.09	3.74	0.41	0.61	0.32	<0.01
S17	6.46	2.94	0.49	0.52	0.06	<0.01
S18	7.09	3.56	0.48	0.57	0.17	<0.01
S19	6.90	3.54	0.37	0.58	0.36	<0.01
S20	6.91	3.81	0.48	0.58	0.18	<0.01
S21	7.55	3.86	0.44	0.59	0.25	<0.01
S22	5.73	3.20	0.52	0.52	0.01	0.22
S23	5.46	2.91	0.51	0.51	-0.01	0.55
S24	7.73	3.72	0.23	0.63	0.63	<0.01
S25	6.55	3.39	0.53	0.55	0.04	<0.01

Table 3.4. Measures of population diversity and differentiation for all 25 sites around Palau calculated on 1188 individual colonies and 11 loci. Standard (F_{ST}) and corrected (F'_{ST}) fixation indicies, inbreeding coefficient (F_{IS}), number of alleles (N), effective number of alleles (N_E), observed (H_O) and expected (H_E) heterozygosities, corrected heterozygosity (H'_T), Nei's inbreeding (G_{IS}) and corrected (G'_{ST}) fixation coefficients and significant levels when appropriate.

F_{ST}	0.30
F'_{ST}	0.08
***p*-value**	<0.01
F_{IS}	0.30
***p*-value**	<0.01
N	13.09
N_E	3.54
H_O	0.90
H_E	0.10
H'_T	0.30
G_{IS}	0.37
G'_{ST}	0.03
***p*-value**	<0.01

Table 3.5. Pairwise F'_{ST} comparison for 25 sites in geographic order around the barrier reef of Palau (top diagonal) and permutation p values (lower diagonal). Nonsignificant F'_{ST} values are shaded.

	S21	S10	S23	S1	S8	S9	S17	S16	S15	S14	S13	S12	S11	S6	S20	S22	S25	S19	S2	S24	S3	S4	S7	S18	S5
S21	--	0.04	0.05	0.05	0.04	0.02	0.04	0.03	0.04	0.04	0.05	0.06	0.01	0.06	0.00	0.04	0.03	-0.02	0.12	0.04	0.12	0.12	0.07	0.02	0.11
S10	0.00	--	0.08	0.05	0.03	0.05	0.11	0.06	0.05	0.05	0.07	0.07	0.05	0.08	0.04	0.05	0.06	0.02	0.13	0.06	0.11	0.10	0.09	0.08	0.09
S23	0.00	0.00	--	0.15	0.14	0.10	0.06	0.12	0.14	0.11	0.11	0.11	0.09	0.14	0.04	0.03	0.00	0.07	0.20	0.12	0.24	0.23	0.19	0.10	0.19
S1	0.00	0.00	0.00	--	0.01	0.05	0.15	0.06	0.03	0.02	0.05	0.10	0.05	0.04	0.07	0.13	0.11	0.07	0.08	0.02	0.02	0.04	0.02	0.11	0.04
S8	0.00	0.01	0.00	0.16	--	0.05	0.15	0.04	0.01	0.02	0.03	0.10	0.05	0.04	0.05	0.11	0.10	0.03	0.11	0.00	0.07	0.07	0.03	0.09	0.08
S9	0.05	0.00	0.00	0.00	0.00	--	0.07	0.04	0.05	0.06	0.09	0.09	-0.02	0.08	0.02	0.07	0.07	0.01	0.15	0.07	0.10	0.08	0.07	0.02	0.09
S17	0.00	0.00	0.00	0.00	0.00	0.00	--	0.09	0.13	0.14	0.16	0.09	0.07	0.16	0.06	0.08	0.05	0.05	0.22	0.15	0.22	0.21	0.18	0.04	0.19
S16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	--	0.02	0.07	0.07	0.11	0.04	0.09	0.05	0.11	0.09	0.06	0.14	0.06	0.08	0.08	0.06	0.05	0.11
S15	0.00	0.00	0.00	0.03	0.16	0.00	0.00	0.05	--	0.06	0.03	0.08	0.05	0.07	0.07	0.11	0.12	0.04	0.09	0.04	0.07	0.09	0.05	0.08	0.12
S14	0.00	0.00	0.00	0.07	0.06	0.00	0.00	0.00	0.00	--	0.04	0.12	0.05	0.02	0.05	0.12	0.07	0.05	0.08	-0.01	0.09	0.06	0.05	0.11	0.05
S13	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	--	0.11	0.09	0.08	0.06	0.10	0.07	0.05	0.11	0.02	0.12	0.11	0.08	0.11	0.14
S12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	--	0.07	0.11	0.08	0.11	0.10	0.05	0.19	0.12	0.16	0.18	0.14	0.11	0.14
S11	0.06	0.00	0.00	0.00	0.00	0.98	0.00	0.00	0.00	0.00	0.00	0.00	--	0.08	0.04	0.08	0.07	0.02	0.15	0.07	0.11	0.10	0.09	0.04	0.11
S6	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00	--	0.09	0.15	0.12	0.07	0.09	0.03	0.09	0.06	0.05	0.14	0.05
S20	0.54	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	--	0.04	0.02	0.00	0.16	0.04	0.16	0.13	0.11	0.03	0.12
S22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	--	0.02	0.04	0.22	0.11	0.20	0.19	0.15	0.05	0.19
S25	0.00	0.00	0.59	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	--	0.04	0.17	0.08	0.20	0.18	0.15	0.06	0.15
S19	0.99	0.09	0.00	0.00	0.02	0.21	0.00	0.00	0.01	0.00	0.00	0.00	0.08	0.00	0.53	0.00	0.00	--	0.16	0.04	0.15	0.13	0.09	0.01	0.13
S2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	--	0.11	0.09	0.12	0.09	0.20	0.11
S24	0.00	0.00	0.00	0.04	0.57	0.00	0.00	0.00	0.01	0.75	0.05	0.00	0.00	0.03	0.00	0.00	0.00	0.03	0.00	--	0.10	0.08	0.05	0.11	0.09
S3	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	--	0.01	0.03	0.17	0.06
S4	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.18	--	0.02	0.14	0.03
S7	0.00	0.00	0.00	0.09	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.06	0.15	--	0.12	0.02
S18	0.03	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.00	0.00	0.00	0.00	0.00	--	0.18
S5	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.14	0.00	--

Table 3.6. Average of summary statistics of indices of genetic diversity within sites grouped by categories of self-seeding and total seeding and permutation test for differences among the groups. OSx-statistic is used to test for significance (Goudet 1995).

Coefficient	**Exposure**					
Self-seeding	Low	Medium	High		**OSx**	***p*-value**
H_O	0.39	0.38	0.33		0.07	0.81
H_E	0.61	0.63	0.64		0.04	0.82
G_{IS}	0.36	0.40	0.48		0.15	0.84
G_{ST}	0.03	0.03	N/A		0.04	0.59
Total-seeding	Low	Med/low	Med/high	High		
H_O	0.43	0.48	0.39	0.32	0.29	0.10
H_E	0.61	0.56	0.60	0.64	0.13	0.21
G_{IS}	0.29	0.15	0.35	0.49	0.62	0.10
G_{ST}	0.03	0.02	0.02	0.03	0.02	0.91

Table 3.7. Average of summary statistics of indices of genetic diversity within sites grouped by exposure zones (SE - southeast, SW - southwest, NE - northeast, NW - northwest) and permutation test for differences among the groups. OSx-statistic is used to test significance (Goudet 1995).

	Exposure Zones					
Coefficient	**SE**	**SW**	**NE**	**NW**	**OSx**	**P-value**
H_O	0.41	0.31	0.39	0.48	0.24	0.02
H_E	0.60	0.64	0.62	0.56	0.13	0.02
G_{IS}	0.32	0.52	0.37	0.14	0.54	0.01
G_{ST}	0.03	0.03	0.02	0.01	0.03	0.27

Figures

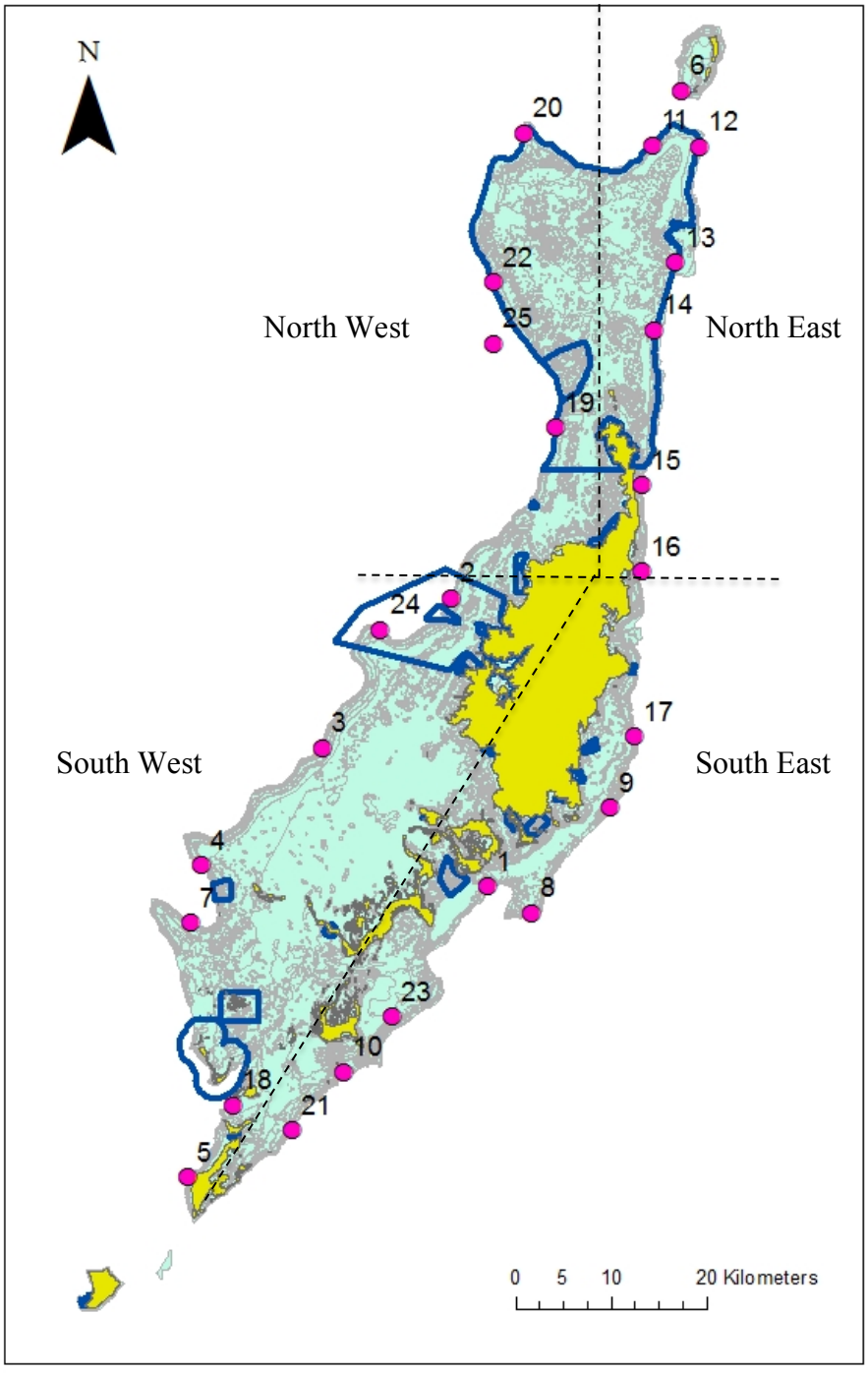

Figure 3.1. Map of Palau and its reefs with 25 sampling location. In blue the delimitations of marine protected areas as defined in 2012. Dashed black lines indicate the division of the reef in exposure zones.

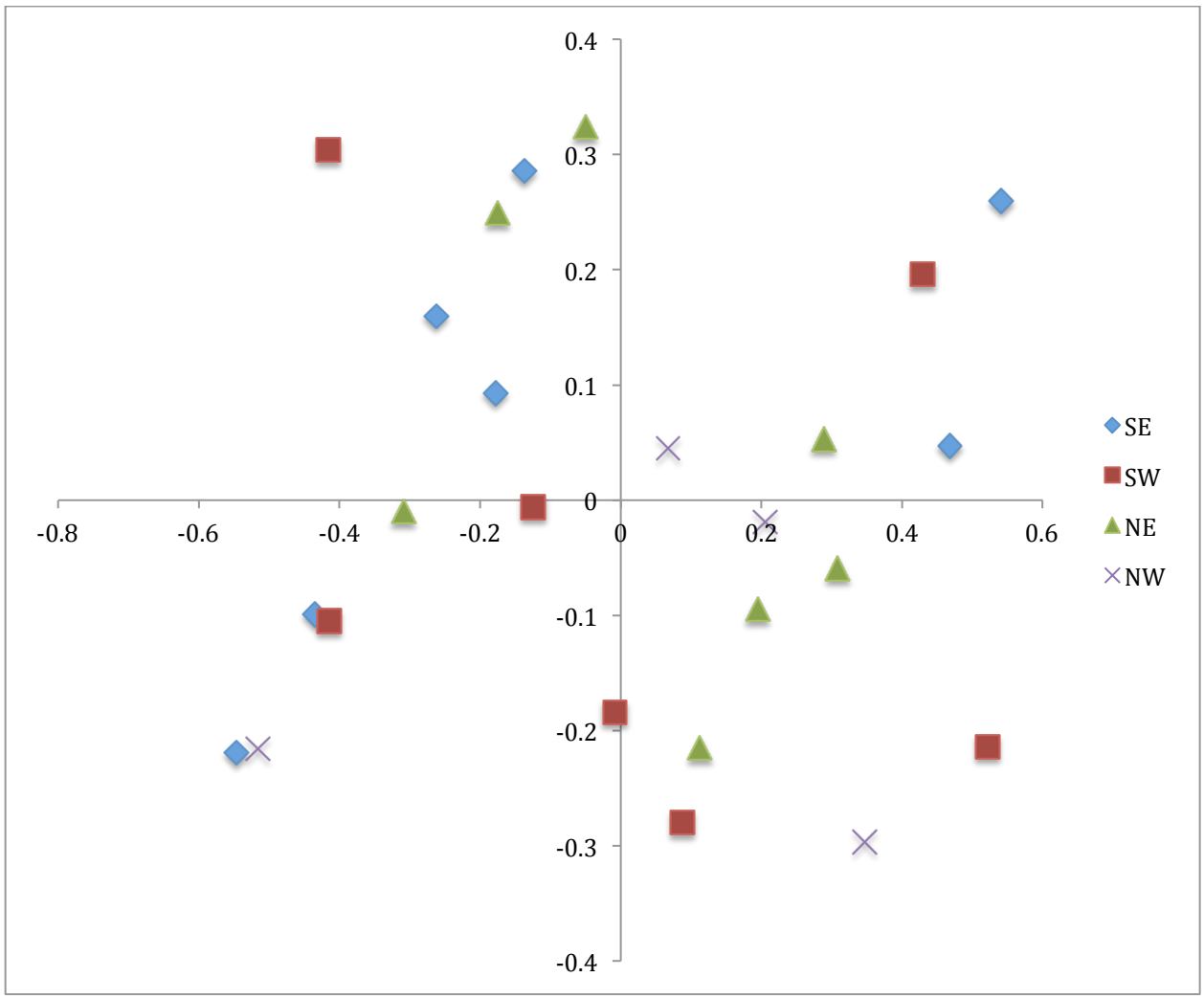

Figure 3.2. Principal Component Analysis of the 25 sites around Palau. The first axis explains 31.9% of the variation and the second axis explains 14.9% of the variation among sites. Sites are grouped by exposure zones (Table 3.7).

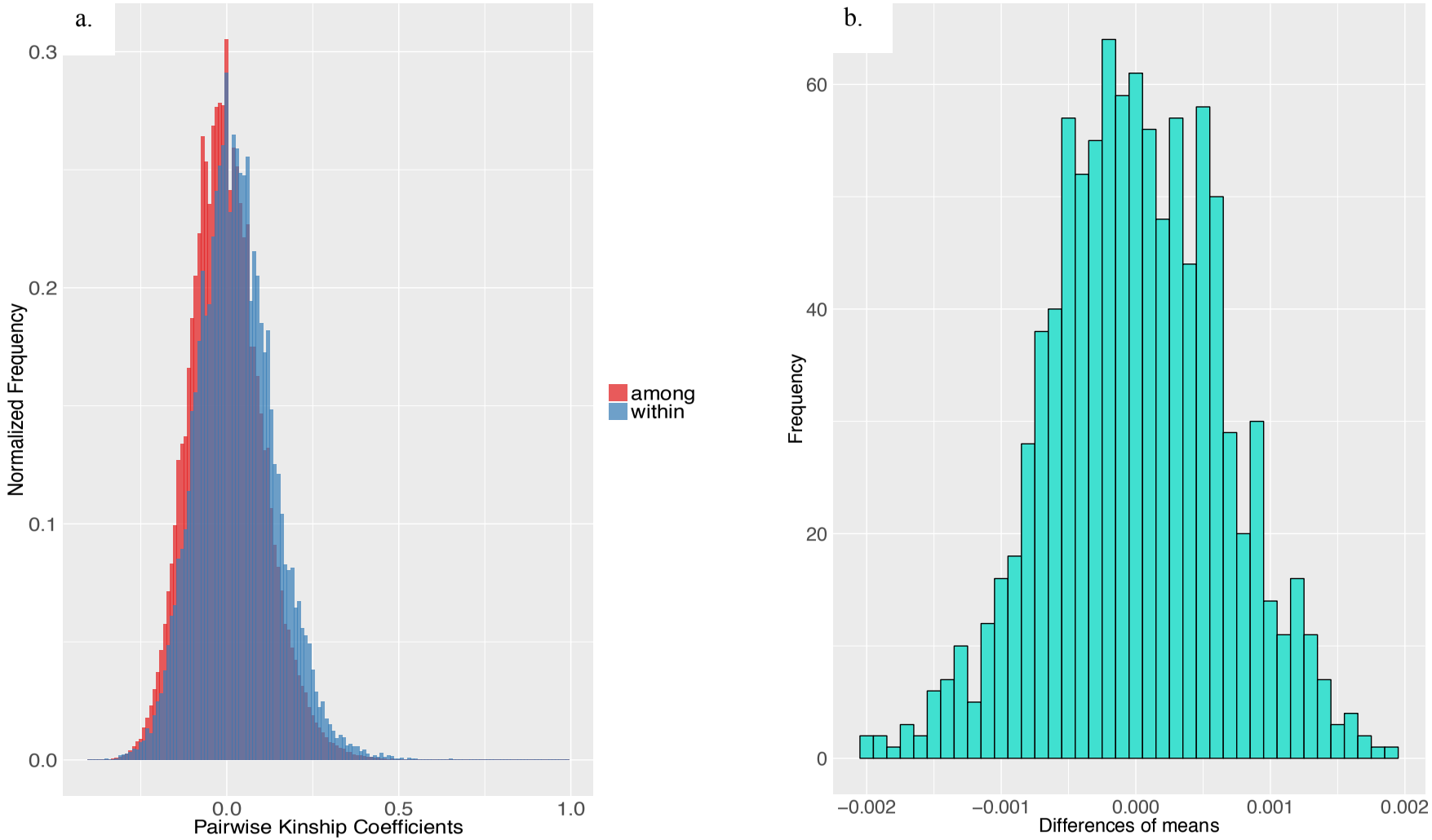

Figure 3.3. a) Histograms of the distribution of the frequency of the pairwise kinship coefficient. In blue, distribution of 27,646 pairwise kinship coefficients within sites. In red, distribution of 677,432 pairwise kinship coefficients between sites. Histograms are plotted with a normalized axis. b) Distribution of the mean differences of the pairwise kinship coefficient within and between sites generated by permutation.

CHAPTER IV. GENETIC NEIGHBORHOODS EXPLAIN GENETIC CHAOTIC PATCHINESS OF *ACROPORA HYACINTHUS*

Abstract

Scale plays a central role in identifying drivers of population structure because the factors influencing patterns and processes of gene flow are frequently scale dependent. To test for population structure and infer dispersal, a majority of population genetic studies use indirect measures of genetic differences such as *F*-statistics. One common approach is to look for evidence of isolation-by-distance, where the farther populations are apart, the more genetically differentiated the populations are. Other approaches include testing the relationship between gene flow and distance. The use of direct measure of genetic differences at the individual level such as kinship or parentage analysis to test for gene flow and dispersal also has been used. Here, I use indirect and direct measures of population genetic differences to infer patterns of gene flow of the table coral *Acropora hyacinthus* at four different scales. Using *F*-statistics, I find chaotic genetic patchiness at the large scale and a lack of isolation-by-distance at all scales. I hypothesize that this pattern indicates that I am not measuring genetic differentiation at the correct scale. A surprisingly clear relationship between mean pairwise kinship coefficients and scale, however, provides an insight of the scale at which dispersal takes place. For Palau, kinship coefficient indicate that population structure of *Acropora hyacinthus* should be observed at a scale of under 400m.

Introduction

One of the principal goals of landscape and seascape genetics is to understand how geographical and environmental factors structure genetic variation at both the population and individual level (Manel et al. 2003; Holderegger and Wagner 2008; Selkoe et al. 2016). Central to identifying drivers of population structure is the question of scale because the factors influencing patterns and processes of gene flow are frequently scale dependent (Levin 1992; Largier 2003; Cowen et al. 2006). For example, in marine systems where most organisms are characterized by a bipartite life cycle with a relatively long pelagic larval phase, it has been hypothesized that long-distance dispersal plays a fundamental role in population structure (Palumbi 1994; Kinlan et al. 2005; Liggins et al. 2013). Dispersal models have therefore focused on modeling large scale processes such as currents and wind patterns that will influence propagule dispersal over long distance and linking genetic differences with broad-scale geographic features (Cowen et al. 2006; Treml et al. 2008; Foster et al. 2012; Wood et al. 2014). This concept, however, is being challenged by an increasing number of studies showing that dispersal is taking place at a much smaller geographical scale and that processes influencing gene flow occur at a finer resolution (Cowen and Sponaugle 2009; Riginos and Liggins 2013; Selkoe et al. 2016).

Drift and mutation cause populations to diverge whereas migration is a homogenizing force and it is the relative importance of each of these processes that dictate the resulting genetic structure in the population. Migration is most common among proximate populations, so that the farther populations are apart, the less migration occurs and the more genetically differentiated the populations become, a pattern called isolation-by-distance (IBD; Wright 1934; Heywood 1991). This pattern, however, is not always clear for marine species, in particular corals, where adjacent populations appear more genetically distinct than populations farther apart and the relationship between genetic structure and distances appears chaotic (Johnson and Black 1984; Ayre and Hughes 2000; Selkoe et al. 2010, 2014; Broquet et al. 2013; Iacchei et al. 2013). Gorospe & Karl (2013) coin the uncoupling of the correlation between genetic and geographic distances as ‘the coral population genetic paradox’ and argue that this paradox may be the consequence of looking for patterns in population genetics at the wrong scale. Testing patterns of genetic

structure at different scales can therefore be a strategic way to better understand mechanisms of dispersal and gene flow for different species.

A large majority of population genetic studies have used indirect measures of population genetic differences not only to understand population structure but also to infer dispersal and gene flow. One common approach is to look for evidence of IBD, where it is expected to find a relationship between linearized genetic differences in populations and distance between these population as first outlined by Wright (1934) for continuously distributed populations. This approach, however, is receiving increasing criticisms, for example Meirmans (2012) demonstrates that hierarchical population structure can create a false signal of IBD. Another approach uses Wright's *F*-statistics (Wright 1949; Weir and Cockerham 1984) to generate a pairwise estimate of gene flow as a function of distance (Slatkin and Maddison 1990; Slatkin 1993; Hellberg 1994, 1995). Slatkin & Maddison (1990) and Slatkin (1995) demonstrate that the regression of pairwise estimates of gene flow on the distance separating populations can be used to identify patterns of dispersal, because under a stepping stone model (Kimura and Weiss 1964), adjacent populations at equilibrium have a predictable slope (Slatkin, 1993). In the case of genetic disequilibrium between gene flow and drift, this relationship will be disrupted. Hellberg (1994, 1995) tested these predictions at different scales against an expected slope of -1 for linearly distributed populations in a one-dimension stepping stone model and a slope of -0.5 for populations spread in a two dimensions stepping stone model and demonstrated that he could identify the mode of dispersal and populations that were not in equilibrium.

The rise of seascape genetics and research questions focusing on patterns of dispersal have increased the use of direct measure of genetic differences at the individual level such as kinship or parentage analysis (reviewed by Selkoe et al., 2016). Kinship coefficients are the probability of identity by descent of homologous alleles sampled randomly from each pair of individuals. Direct analysis may identify dispersal and recruitment patters that cannot be detected using traditional *F*-statistics, yet relatively few studies used both measures to study dispersal and population structure (Loiselle et al. 1995; Vekemans and Hardy 2004; Iacchei et al. 2013). Additionally, kinship coefficients

do not require assumptions of equilibrium and provide an unique measure that is scale independent (Iacchei et al. 2013).

In this study, I use indirect and direct measures of population structure to infer patterns of gene flow of the table coral, *Acropora hyacinthus*, at four different scales. 1. Large scale is defined by the distance between the islands of Yap, Ngulu and Palau, separated by 160 to 550km (Fig. 4.1a) 2. Medium scale is defined as the distance between sites on the same island separated by 5 to 150km (Fig. 4.1b, c, d, Fig. 4.2). 3. Small scale is defined by distances between individuals within a site separated by 5 to 400m. 4. Fine scale is defined by individuals within a single belt transect separated by less than 100m (Fig. 4.2). Our goal is to identify the scales at which a genetic pattern either between individuals or populations is detected and to compare this pattern across the spatial scales that were sampled. I hypothesize that finding a pattern of chaotic genetic patchiness at a particular scale indicates that genetic structure is not measured at the correct scale.

Methods

Study species

Acropora hyacinthus is a widely distributed table coral that can be found on shallow reefs of Palau between 3 and 10m but is rare or absent on the patch reefs, fringing reefs and lagoon (Bruno et al. 2001). It reaches maturity around four to five years of age (Wallace 1985), which corresponds approximately to a 15 - 20 cm colony (Guest et al. 2005; Baria et al. 2012). Although *A. hyacinthus* can reproduce asexually through fragmentation, previous studies show that very few clones have been found in the field (Ayre and Hughes 2000; Márquez et al. 2002). *A. hyacinthus* is a hermaphrodite broadcast spawner with a larval pelagic duration time of 90 days under laboratory conditions (Márquez et al. 2002). It is presently one of the dominant coral species growing on Palau's barrier reef (Golbuu et al. 2007; Victor et al. 2009), however, *A. hyacinthus* suffered heavy mortality from a 1998 bleaching event in Palau, virtually disappearing from the atoll (Bruno et al. 2001).

Sampling locations and methodology

To study population structure at all four scales, coral colonies in Yap, Ngulu Atoll and Palau (Fig. 4.1a, Table 4.1) and transects in Palau (Table 4.1) were collected. Sampling in Yap, Ngulu Atoll and Palau (Fig. 4.1a, Table 4.1) was carried out by two different laboratories in different years. In 2009 – 2012 Davies et al. (2015) sampled Yap and Ngulu. Briefly, at three sites on the barrier reef of Yap and a single site on the barrier reef of Ngulu (Fig. 4.1c, d), approximately 50 colonies (>2m apart) were randomly sampled using SCUBA or snorkeling. One small (~2 cm^3) branch tip was collected, preserved in 96% ethanol and stored at 20 °C (Davies et al. 2015). In Palau, sampling took place in February and May 2012 at 25 sites along the outer barrier reef at a shallow depth (<10m) using SCUBA (Fig. 4.1b, 2). Sites were selected in each of the four exposure zones around Palau, northeast (NE), northwest (NW), southeast (SE) and southwest (SW). A total of 1200 x 2cm^3 colony tips were collected by sampling haphazardly 48 colonies (>2m apart) of *A. hyacinthus* in areas of 4 x 200m^2 at each of these 25 sites. At six of the 25 sites, in a belt transect of 2 x 100m^2, all colonies of *A. hyacinthus* that could be identified reliably (of a size of more than 5cm in diameter) were additionally exhaustively collected. Each colony in the transect was photographed, measured and given a position on an X and Y axis with the bottom left corner of the belt transect as the origin. One small branch tip per colony (<2cm^3) was cut and preserved in salt-saturated DMSO at room temperature (Gaither et al. 2011).

DNA extraction and sequencing

A detailed description of DNA extraction and sequencing is described in (Cros et al. 2016a, 2016b). Briefly, genomic DNA was amplified at eleven microsatellite loci (Appendix 1) with colony identification (ID) tags (Appendix 2) and pooled by sites. I used a different ligated Illumina adaptor (Illumina Inc., Hayward, CA, USA) for each colony from the same collection site to generate a library with a unique ID per individual per site. Libraries were sequenced on an Illumina MiSeq at the Hawaii Institute of Marine Biology, and each samples could be assigned to an individual and site based on the unique barcode ID.

Data processing

I used the bioinformatics pipeline in Cros et al. (2016a) to process the raw sequences.

In brief, the sequences were demultiplexed by site, merged, separated according to primer and colony and trimmed for low quality sequences. Sequences were collapsed into unique sequences and used depth to apply filters developed in python (https://github.com/annickcros/Ahyacinthus-filters.git) for PCR and sequencing artifacts. Simple tandem repeats (STR) were separated from flanking regions with emboss: etandem and used to generate genotypes. Data were transformed in genodive v. 2.0b27 (Meirmans 2014) file format using formatting as described in Cros et al. (2016a). The final analysis was carried out on two different datasets. First, I used 11 loci (Table 4.2) to calculate measures of population differences after eliminating loci with 15% or greater missing data for all sites overall. Second, I used a dataset with nine loci (Table 4.2) to calculate kinship coefficients after eliminating two additional loci with 15% missing data among samples from the transects. The final number of colonies per sites analyzed for each locus varied between 37 and 48.

Analysis

Descriptive analysis

I used 11 loci in the first dataset of population measures (global F'_{ST}, pairwise F_{ST}, pairwise F'_{ST}, Mantel test: Table 4.2). I used GENODIVE V.2.27 to test for clones and to run descriptive analysis and reported the number of alleles, the effective number of alleles and indices of genetic diversity at each of the 29 sites, as well as observed (H_O), expected (H_E) and corrected heterozygosity (H'_T), inbreeding coefficient (G_{IS}) and Nei's fixation index G_{ST} (Table 4.2).

Measure of genetic structure at four scales

To understand the mechanisms of dispersal at the four different scales, I used both an individual and population-level approach. I used *F*-statistics, as the measures of population structure and kinship coefficients (Loiselle et al. 1995) as the measure of genetic difference between individuals. *F*-statistics were measured at the large and medium scale where populations were defined as sites. Kinship coefficients were calculated and compared at all four scales.

Large & medium scales: Population structure, isolation-by-distance and gene flow

To understand population structure at the large and medium scales, I created a matrix of pairwise F'_{ST} among sites and tested for significance in GENODIVE with 10,000 permutations (Appendix 10). To test for IBD, I created a matrix of pairwise F_{ST} values and a matrix of pairwise distances around the barrier reef among sites around the reef of Palau of sites from S1 to S25, Yap sites S27, S29 and S30 and Ngulu site S28 (Appendix 10). I tested for IBD with IBD Web Service (http://ibdws.sdsu.edu) using log-log transformations with 100,000 permutations at two scales, 1) Palau only and 2) Palau, Ngulu and Yap. I compared results with stratified Mantel tests controlling for clusters as per Meirmans (2012) using islands as the strata factor in GENODIVE V.2.27 with 100,000 permutations.

I tested gene flow over the large and medium scale using R v.3.2.3 (Team 2008) plotting the regression of gene flow, the pairwise estimate of *Nm* and geographic distance in km. I used a log-log transformation of both genetic and geographic distance as in previous studies (Slatkin and Maddison 1990; Slatkin 1993; Hellberg 1994, 1995). To calculate the 95% confidence interval (CI) of the slope I used a bootstrap permutation with 1,000 permutations in R (package boot v 1.3-18).

Large, medium, small and fine scale: pairwise kinship coefficients

Kinship coefficients were calculated in GENODIVE relative to all colonies of *A. hyacinthus* available: Palau sites S1 through S25 (excluding S19 with too many missing alleles), Yap sites S27, S29, S30, Ngulu site S28, Palau transects T130, T200, T210, T220, T240 and T250. Overall, I estimated pairwise kinship among a total of 1318 colonies.

To compare the distribution of kinship coefficient at the four scales, I used R (package ggplot2 v.2.1.0) and plotted the mean and confidence interval of pairwise kinship coefficients at each of the four scales (Fig. 4.3). I performed a Kruskal-Wallis test in R to see if there were significant differences between means. The Kruskal-Wallis test is a non-parametric test, which can be used when the data do not meet assumptions of normality and/or heteroscedasticity and with unequal sample sizes. I used the Dunn Test (packages FSA v.0.8.8 and dunn.test v.1.3.2) as a *post hoc* test (Appendix 12).

Violin plots of the pairwise kinship coefficients (package plotrix v. 3.6-2) were plotted at different scales to look more closely at the effect of the tails of the distributions on population structure (Fig. 4.4).

To evaluate the effect of distance on kinship I plotted the proportion "related" and "unrelated" pairwise kinship coefficients against scales (as a proxy for distance) in a mosaic plot (Fig. 4.5). Mosaic plots show independence between categories when the boxes all have the same areas across categories. To create the "related" and "unrelated' bins, I first binned counts of closely related colonies according to specific levels of kinship coefficients using Loiselle et al. (1995) coancestry coefficients (k; full-sib = 0.25, half-sib = 0.125). Following methods from Iacchei et al. (2013) I generate the following bins: 'nearly identical': $k > 0.375$; 'full-sib': $0.375 > k > 0.1875$; 'half-sib': $0.1875 > k > 0.09375$; and 'quarter-sib': $0.09375 > k > 0.047$. After initial analysis I simplified these bins to "related" for kinship $>= 0.09375$ and "unrelated" for kinship < 0.09375. To test the relationship between scales and kinship statistically I used a general linear model and a Tukey *post hoc* test in R (package multcomp v 1.4-6) (Fig. 4.6).

Fine scale: Spatial autocorrelation and effect of colony size on kinship

I tested for autocorrelation in the five transects T130, T200, T220, T240 and T250 using Moran's I in SPAGEDI V1.3 comparing two matrices: pairwise kinship coefficients (Loiselle et al. 1995) and relative X, Y distances of each colony. Moran's *I* evaluates whether the pattern expressed is clustered, dispersed, or random. I plotted the mean pairwise kinship coefficient (averaged over each distance bin) over the 95 % confidence interval (CI) of the permuted value (Appendix 11). When the mean falls outside of the 95% CI of the permuted value the null hypothesis that the observed mean and the permuted mean are the same is rejected and there is spatial autocorrelation at the scale of the distance bin. Transect T210 did not have a complete dataset of distances and was excluded from this analysis.

To check if size could be a proxy for parent/offspring at the scale of a transect, I tested the effect of the colonies size on kinship coefficients with a Mantel test in R (package Vegan v.2.3-5) for five of the six transects. To build the size matrix, I looked at absolute

pairwise differences between colonies. I ran a parentage analysis in the parentage analysis software SOLOMON using sizes >=20cm as parents and <20cm as potential offspring, with a size gap of approximately 2 to 5cm.

Results

Descriptive statistics

I used GENODIVE v. 2.0b27 to test for clones and did not find any. The rest of the analysis is conducted on single ramets. I genotyped between 37 and 178 colonies at each site (Table 4.1) for each of our eleven microsatellite loci. At each site, the effective number of alleles per locus varied between 1 and 9.6 (Table 4.2). Observed heterozygosity (H_O) ranges from 0.007 to 0.69 and expected heterozygosity (H_E) from 0.007 to 0.91 and all loci are out of Hardy Weinberg Equilibrium (HWE). The inbreeding coefficients were all significant ($p<0.01$, Table 4.2).

Large and medium scale: Population structure and isolation-by-distance

Pairwise F'_{ST} values (Appendix 10) show significant structure among sites around Palau (see Cros et al 2016b for a discussion on this) and among sites on each of the three islands.

I tested to see if there was isolation by distance at the large and medium scales. Results from IBD web service shows isolation by distance at the large scale between Yap, Palau and Ngulu ($r^2=0.047$, $p<0.01$). When I repeat the Mantel test in GENODIVE stratifying by island, the p value is no longer significant ($p=0.231$) showing that there is hierarchical clustering at the island level (Meirmans 2012). I repeated the test at the medium scale, for sites around Palau and found no signs of IBD.

I tested gene flow against distance using gene flow and the distance between sites at two different scales (Appendix 13). Overall, from short to long distances, I find a slope of -0.18 (95%CI=-0.25 – -0.12) with $r^2= 0.08$. At the medium scale, for sites in Palau I find a slope of -0.12 (95%CI=-0.25 – -0.02) with $r^2=0.02$ and for Yap I find a slope of -0.50 (95%CI=-0.74 – -0.48) and $r^2=0.99$. Only the slope for Yap matched the prediction of -0.5 for a stepping stone model in equilibrium but with n=3 this result needs further testing. At the large scale, for pairwise sites between Palau, Yap and Ngulu, I find a slope

of 0.5 (95%CI=-2.06, 0.55) with r^2=0.06. The positive slope at the large scale is surprising, describing an increase in gene flow with distance. This indicates that there is less gene flow between Yap and Ngulu than between the more distant Palau and Yap.

Large, medium, small and fine scale: Pairwise kinship coefficients

To compare genetic structure among the four scales, I plotted the mean and confidence interval of the mean of each distribution (Fig. 4.3), clearly showing an effect of scale on pairwise kinship coefficients. The Kruskal-Wallis test showed that all means were significantly different from each other (χ^2=5600, df=8, p<0.01) and the Dunn *post hoc* test was significant for all pairs except for two. The mean of the fine scale samples within transects and mean of the medium scale samples among Yap were similar as well as the mean of medium scale among Yap and the mean of small scale within Palau (Appendix 12). This highlights the higher values of kinship for colonies in Yap. The mean between Yap and Ngulu stands out at 0.027 because it is much higher than the other large-scale pairwise comparisons.

Although the means were all significantly different, the violin plots (Fig. 4.4) show that the distributions overlap at all four scales: fine (within transects), small (within sites), medium (between sites) and large (between islands) and for all three islands. The line indicates the divide between "related" and "unrelated" (k=0.0937) and helps to identify the sites that display the highest frequency of related colonies. Ngulu displays the highest mean pairwise kinship coefficients, but only one site was sampled there. Pairwise kinships at fine and small scale have a more positive distribution than those at medium and large scale. Palau has the longest positive tail indicating that it has some of the highest pairwise kinship coefficients. Overall, as distance between colonies increases, the distribution of pairwise kinship coefficients decreases.

I plotted a mosaic plot of the kinship coefficients binned to related and unrelated individuals against sites in Palau (Fig. 4.5). The mosaic plot shows that there is a difference in the ratio of related to unrelated pairwise colonies at different scales with the largest proportion of related colonies within transects (wT) and within sites (wP). Palau as compared to Ngulu (PN) had the fewest related colonies. The general linear model and Tukey test shows that differences among ratios of related to unrelated pairwise colonies

in the mosaic plot are statistically significant between all scales, and that there is a significant effect of scale on the relationship. I plotted the proportions of related to unrelated pairwise colonies (Fig. 4.6) and show that there is an increase of relatedness with scale except between the fine and small scale.

Fine scale: Spatial autocorrelation and effect of size on kinship

I tested for autocorrelation in the five transects T130, T200, T220, T240 and T250 using Moran's *I* in SPAGEDI V1.3 comparing two matrices: pairwise kinship coefficients (Loiselle et al. 1995) and relative X, Y distances of each colony. I found significant autocorrelation in only three transects T130, T200 and T210 for three bins for distances from 1 to 7m (Appendix 11). There are too few autocorrelation to lead to the conclusion that there is spatial autocorrelation at the scale of transects. Overall there is not a strong pattern of spatial autocorrelation between kinship and distances between colonies at scales >7m.

When testing for correlation between kinship and absolute differences in colony sizes I found no correlation ($p>0.05$). I also found no parent/offspring combination in the parentage analysis.

Discussion

Using indirect and direct measures of population genetic differences to infer patterns of gene flow of the table coral *Acropora hyacinthus* at four different scales provides a comprehensive idea of the processes that influences the genetic structure of populations of *A. hyacinthus* on the reef. At the large scale there is little evidence of connectivity for *A. hyacinthus*. Instead, there is a strong pattern of population genetic difference between Yap, Palau and Ngulu. The little exchange of larvae is confirmed by low means for the distributions of pairwise kinship coefficients of colonies between islands. The analysis of gene flow displays low values of gene flow between Yap, Palau and Ngulu, but surprisingly it also shows a positive relationship between gene flow and distance. Sites between Palau and Yap exhibit higher gene flow than sites between Yap and Ngulu, which is unexpected for a couple of reasons. First, the mean pairwise kinship coefficient between colonies in Yap and Ngulu is higher than between Palau and Yap although it remains under the half-sibling cut-off. Second, because of the close proximity of Ngulu

and Yap, about 100km south-southwest of Yap, which is about half the distance between the Yap and Palau and in the path of potential larval dispersal from Yap to Palau. The overall slope of the linear regression between all scales from distances within an island to distances between islands indicates, however, a negative slope of -0.18 as expected. This result suggests that there is some factor influencing genetic differentiation between Yap, Palau and Ngulu that is more important than the effect of distance.

Similarly to the large scale, at the medium scale, sites around Palau show significant genetic structure with no signs of isolation-by-distance. In the case of Palau, gene flow decreases with distance but with a small regression coefficient explaining very little of the variation observed. The mean pairwise kinship coefficients are low (Fig. 4.3, 4.4) indicating little dispersal of colonies at the scale of 150km. Again, there are no indicators of patterns of dispersal or genetic structure at this scale. Yap, on the other hand, shows similar population structure as Palau between its three sites (Appendix 10) yet exhibits a mean kinship coefficient significantly higher than that of Palau, which suggests that processes of dispersal take place at the medium scale for Yap.

The proportions of full and half siblings and the mean kinship for Palau are the highest at the small scale, within sites. Although the effect of distance on genetic differences could not be studied at this scale because all colonies had the same GPS coordinate, one hypothesis to explain high kinship between individuals is that larval dispersal may be taking place at distances under 400m. This short dispersal distance is unexpected for a coral with a long pelagic duration time, but which matches well descriptions of dispersal in recent literature (Hughes et al. 2000; Hellberg 2007; Cowen and Sponaugle 2009; Gorospe and Karl 2013; Kinlan et al. 2016). Essentially, the realized dispersal distance is much smaller than the potential distance based on pelagic larval duration. Another possible hypothesis is that the high mean kinship coefficients is the result of patchy recruitment: larvae are transported and recruit together as a cohort (Selkoe et al. 2006; Broquet et al. 2013; Riginos and Liggins 2013). This would mean that larvae disperse at a larger distance but exhibit patterns of genetic differences at a smaller scale.

The fine scale shows no convincing signs of spatial autocorrelation and the mean kinship coefficient is lower than for the small scale. Both of these results may be due to boundary effects, which are common issues in spatial analysis where the shape and size of the boundary of the experiment affect the observation. A 100x2m^2 belt transect may be too small and the wrong shape to capture the true spatial relationship between colonies (Gorospe et al. 2015), which might be better resolved with a polar coordinates design (Baums et al. 2006a). The chaotic patchiness at the finest scale indicates zooming in too closely to be able to observe factors that will structure populations of *A. hyacinthus*.

Like many previous studies of population genetic structure in corals, when looking for a relationship between genetic differences and distances at four spatial scales, there was no indication of IBD at any of the scales using *F*-statistics as a measure of genetic difference. In contrast, there was a striking relationship between geographic scale and mean kinship coefficients. The difference between *F*-statistics and kinship coefficient is due to the clustering of closely related colonies into genetic neighborhoods where inbred family groups have very little mixing between them and where gene flow, mutation and migration are no longer in equilibrium. As *F*-statistics no longer measure population structure based on the probability of identity-by-descent, finding the patterns dictated by populations in equilibrium can no longer be expect. This is reflected in the pattern of chaotic genetic patchiness of *A. hyacinthus* at the medium scale for Palau and in the slope of gene flow at the medium and large scale.

Tables

Table 4.1. Sampling sites: main island group, date of collection, GPS coordinates, and number of samples genotyped. * indicates that samples were collected by Davies et al. (2015).

Island	Site	Date of collection	Latitude	Longitude	# individuals
Palau	S1	14.02.12	7.287N	134.502W	48
Palau	S2	18.02.12	7.561N	134.468W	47
Palau	S3	16.02.12	7.418N	134.345W	48
Palau	S4	17.02.12	7.307N	134.231W	47
Palau	S5	20.02.12	7.011N	134.218W	44
Palau	S6	11.03.12	8.042N	134.686W	46
Palau	S7	22.02.12	7.252N	134.220W	45
Palau	S8	21.05.12	7.261N	134.544W	48
Palau	S9	21.05.12	7.362N	134.619W	48
Palau	S10	22.05.12	7.111N	134.366W	48
Palau	S11	23.05.12	7.990N	134.659W	48
Palau	S12	23.05.12	7.988N	134.703W	48
Palau	S13	24.05.12	7.878N	134.681W	48
Palau	S14	25.05.12	7.815N	134.660W	47
Palau	S15	26.05.10	7.667N	134.649W	48
Palau	S16	26.05.11	7.586N	134.649W	48
Palau	S17	26.05.12	7.429N	134.642W	48
Palau	S18	28.05.12	7.079N	134.261W	48
Palau	S19	29.05.12	7.722N	134.567W	48
Palau	S20	31.05.12	8.001N	134.536W	48
Palau	S21	01.06.12	7.055N	134.318W	48
Palau	S22	02.06.12	7.860N	134.508W	48
Palau	S23	04.06.12	7.163N	134.412W	48
Palau	S24	05.06.12	7.530N	134.401W	48
Palau	S25	06.06.12	7.801N	134.508W	48
Yap*	S27	2009	9.574N	138.203W	37
Yap*	S29	2009	9.5631N	138.848W	48
Yap*	S30	2009	9.4348N	138.339W	47
Ngulu*	S28	2009	8.3033N	137.4885W	46
Palau	T130	24.05.12	7.878N	134.681W	93
Palau	T200	31.05.12	8.001N	134.536W	178
Palau	T210	01.06.12	7.055N	134.318W	66
Palau	T220	02.06.12	7.860N	134.508W	108
Palau	T240	05.06.12	7.530N	134.401W	87
Palau	T250	06.06.12	7.801N	134.508W	61

Table 4.2. Measures of population differentiation. Range of length of microsatellites (**nt**), number of alleles (***A***), effective number of alleles (A_E), observed heterozygosity (H_O), expected heterozygosity (H_E), total heterozygosity (H_T), adjusted total heterozygosity (H'_T), inbreeding coefficient (G_{IS}) and Nei's corrected fixation index (G_{ST}) for each locus in the entire dataset.

Locus	**nt**	***A***	A_E	H_O	H_E	H_T	H'_T	G_{IS}	G_{ST} **(Nei)**	***p***
loc1	28-36	4	1.007	0.007	0.007	0.007	0.007	-0.011	0.009	<0.01
loc3	21-36	10	2.928	0.659	0.667	0.687	0.688	0.012	0.03	<0.01
loc4	15-75	26	9.695	0.651	0.914	0.928	0.928	0.288	0.015	<0.01
loc5	57-90	11	2.276	0.373	0.571	0.612	0.613	0.347	0.068	<0.01
loc6	36-45	5	1.742	0.319	0.434	0.455	0.455	0.264	0.048	<0.01
loc8	39-108	14	4.365	0.668	0.782	0.804	0.804	0.146	0.027	<0.01
loc11	12-44	7	2.064	0.418	0.524	0.574	0.575	0.201	0.09	<0.01
loc12	36-93	30	5.582	0.488	0.841	0.859	0.86	0.42	0.022	<0.01
loc13	54-81	14	3.763	0.505	0.751	0.776	0.776	0.327	0.033	<0.01
loc14	20-84	24	2.89	0.456	0.666	0.687	0.687	0.315	0.032	<0.01
loc16	32-56	7	1.461	0.16	0.322	0.346	0.347	0.503	0.072	<0.01

Figures

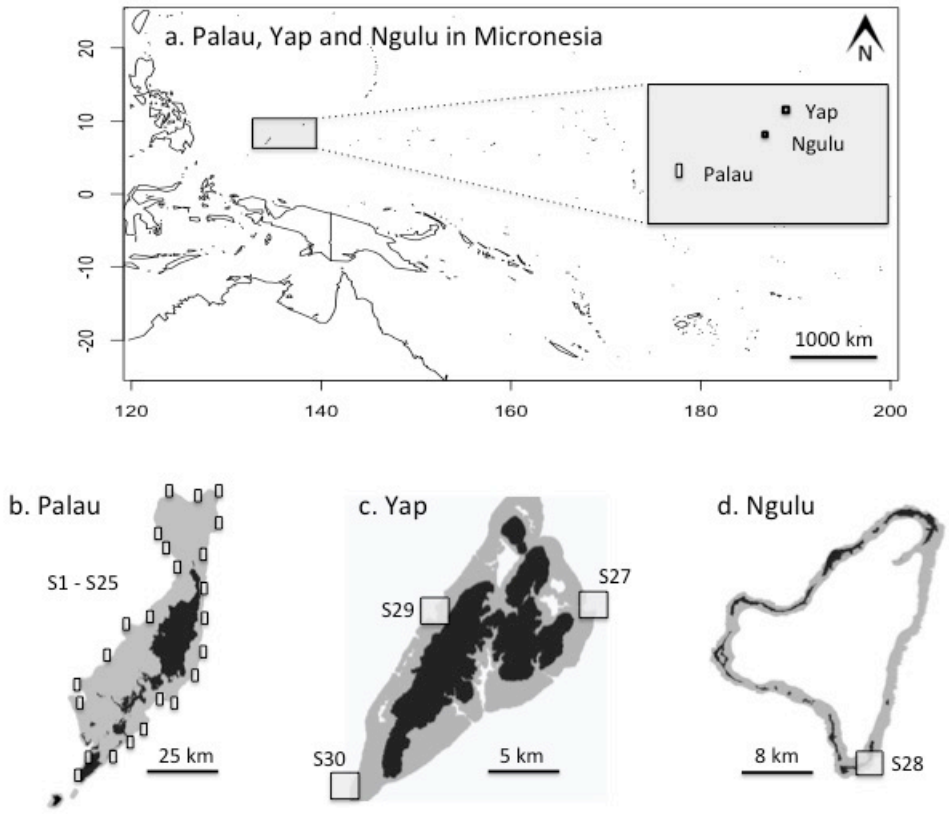

Figure 4.1a) Geographic position of Palau, Yap and Ngulu Atolls in Micronesia. Collection sites in b) Palau (25 sites and 6 transects) c) Yap (3 sites) and d) Ngulu Atoll (1 site).

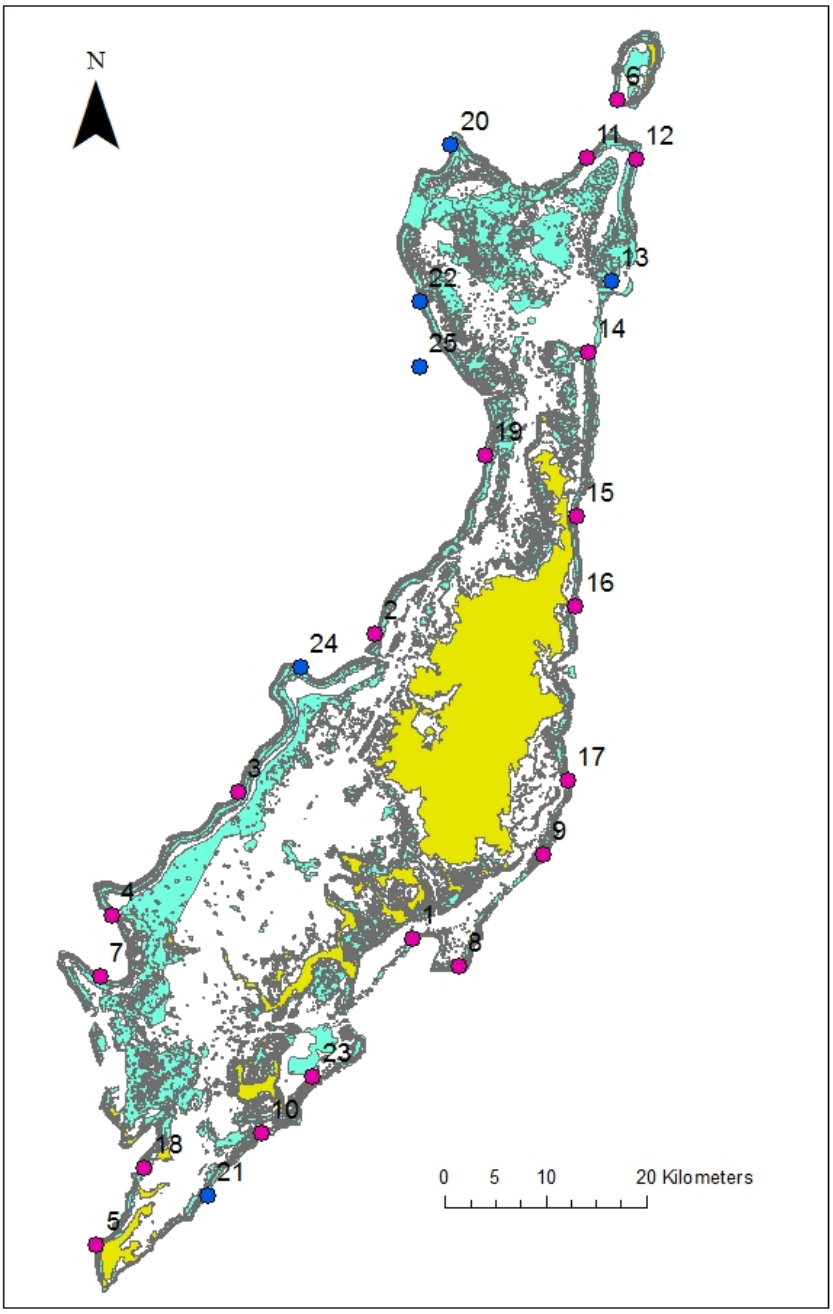

Figure 4.2. Map of all 25 sampling sites in Palau. In blue, sites with transects.

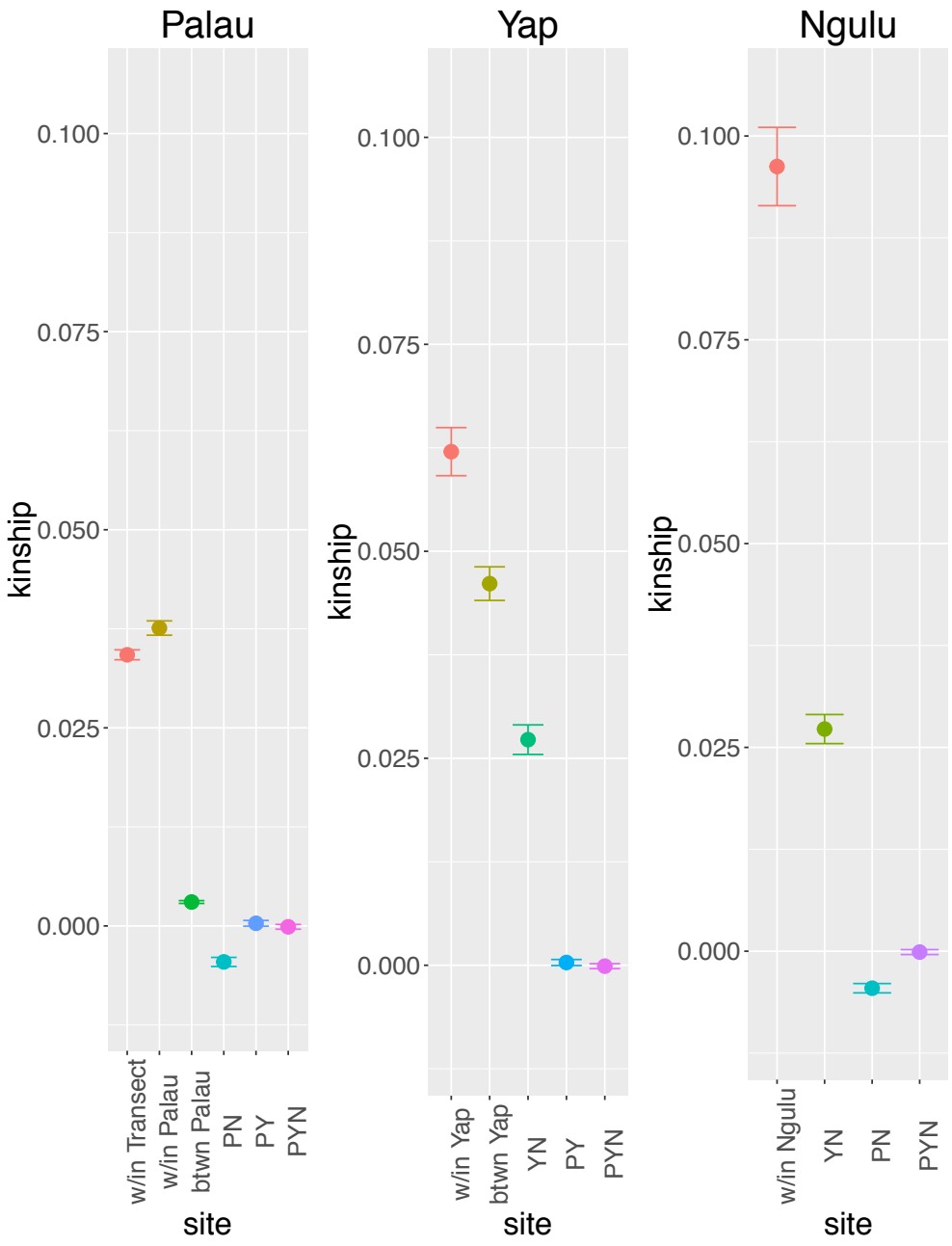

Figure 4.3. Mean and confidence interval of pairwise kinship coefficient by islands at different scales and sites.

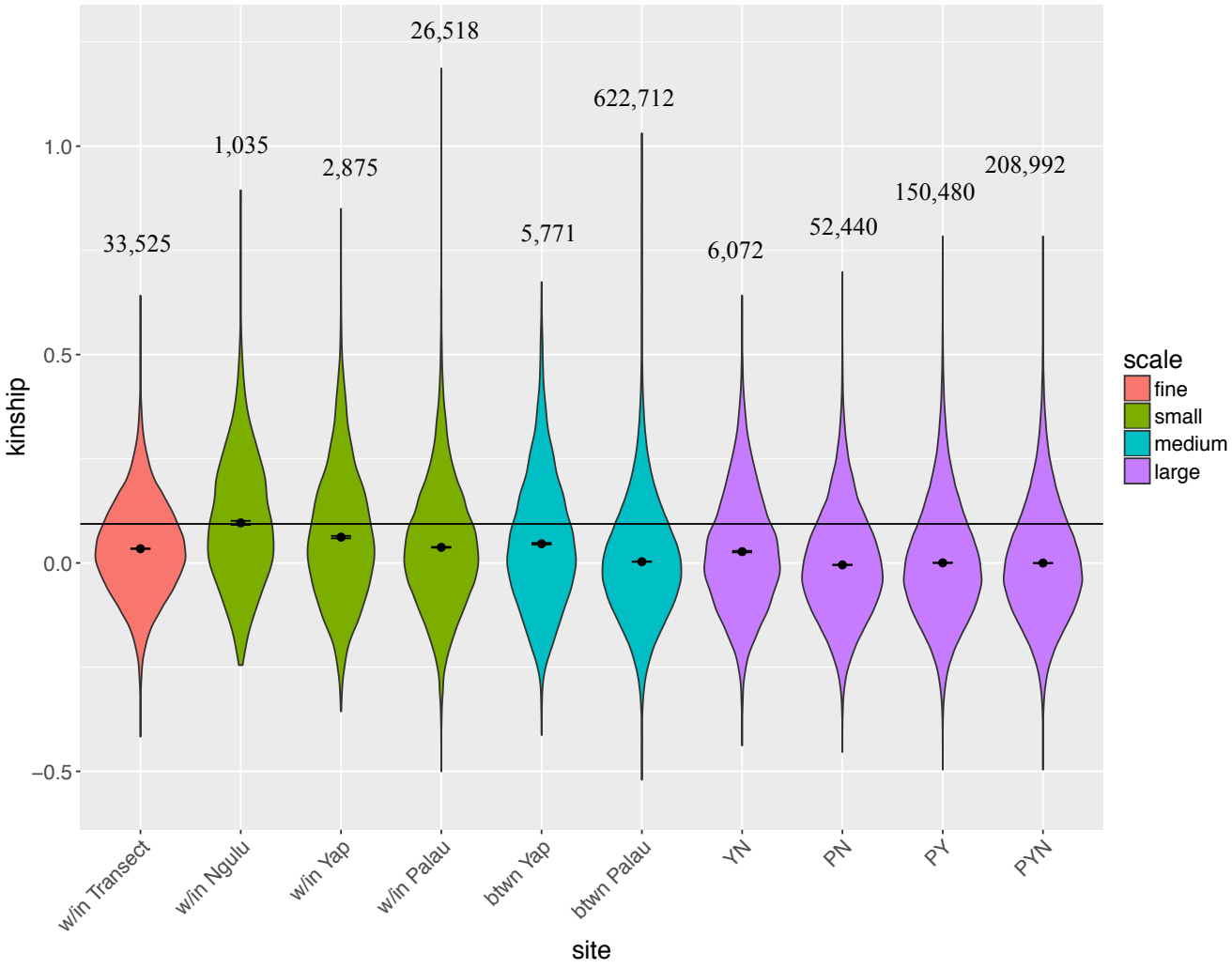

Figure 4.4. Violin plot of the distribution of pairwise kinship coefficients at all scales divided by sites. In red: large scale represented by the distance between colonies on different islands; in green: medium scale represented by the distance between colonies in different sites in the same island; in turquoise: small scale represented by the distance between colonies in the same sites and in purple: fine scale represented by distance between colonies in a transect. In black the mean and confidence interval of the mean for each distribution. The black line indicates the division between "related" and "unrelated" (k<=0.09375).

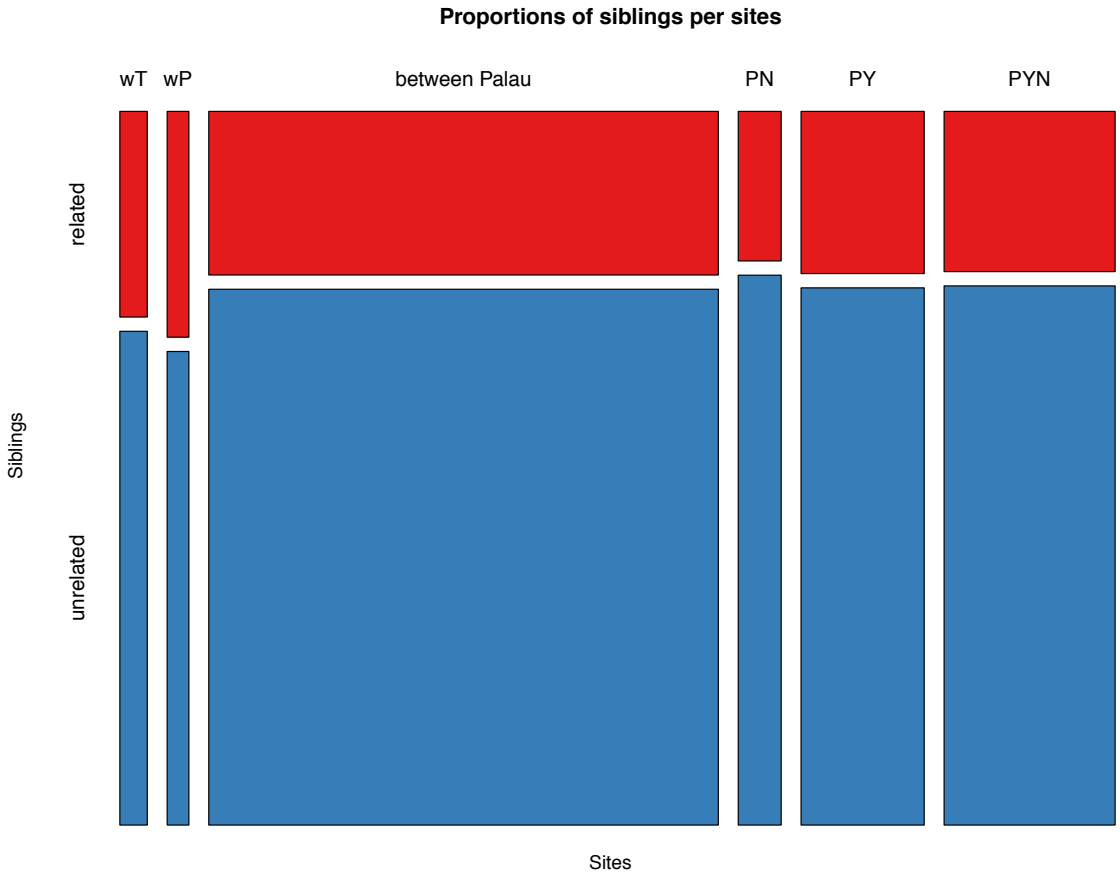

Figure 4.5. Mosaic plot of the abundance of related and unrelated colonies: in red the related colonies with k>=0.09375 and in blue unrelated colonies with k<0.09375 for pairwise sites in Palau: PYN – between coral colonies in sites on different islands, PY – between coral colonies in Palau and Yap, PN - between coral colonies in Palau and Ngulu, between Palau – between colonies in sites in Palau, w.P – between colonies within sites in Palau and w.T -between colonies within transects in Palau.

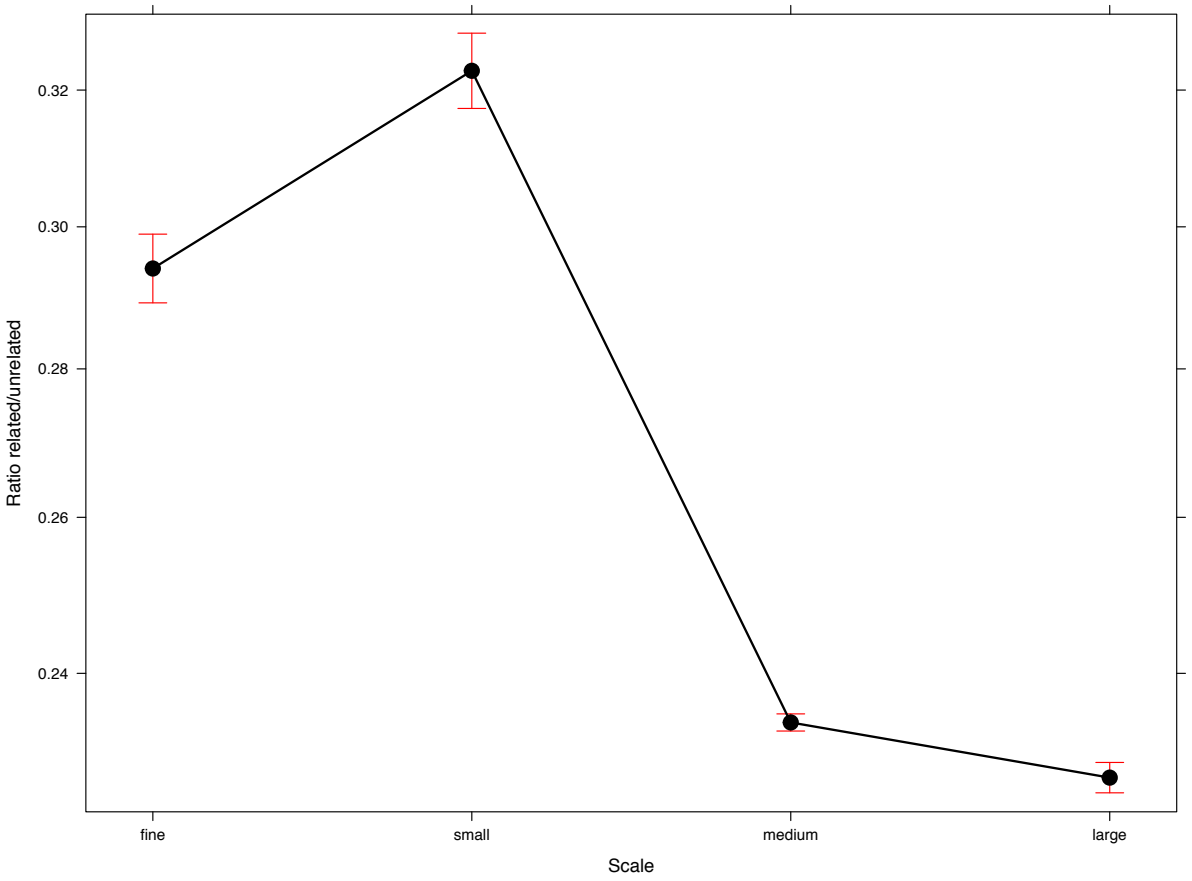

Figure 4.6. Plot of ratio of related/unrelated pairwise individuals against scale.

CHAPTER V. RECOVERY AND RESILIENCE OF THE REEFS OF PALAU

Abstract

Understanding the recovery process of coral reefs can provide information on resilience. The Palau International Coral Reef Center has been monitoring the recovery of the reefs of Palau after the 1998 bleaching event. To understand the dynamic between algae and corals and to describe the succession of coral assemblage on the reef, I use monitoring data collected over a period of seven years at sixteen sites and at two depths. Changes in benthic cover plotted in a triangle plot over time show that algae did not colonize reefs when coral cover was low. A mixed linear model shows that depth and exposure are two significant factors in the recovery patterns coral cover. Sites in protected bays explain most of the variation, showing very little impact from the 1998 bleaching event and consequently very little recovery. The changes in coral assemblage through the recovery process described by genera (diversity) and by bleaching susceptibility show an increased of diversity at all sites and an increase in the cover of resistant corals with time. We recommend dividing the reef into different management units with different management plans according to exposure. The priority for barrier reefs is to manage overfishing whereas for bays it is to manage for impacts from proximity with land.

Introduction

The high mortality of coral reefs worldwide during the 1998 bleaching event played an important role in alerting scientists and conservationists to the impact of climate change. Since then, research efforts have focused on understanding factors that enhance the ability of coral reefs to resist or to recover from thermal stresses and provide solutions to build resilience. Fewer projects, however, have focused on understanding the recovery process itself (Bellwood et al. 2004; Graham et al. 2011; Johns et al. 2014) such as the evolution of coral communities with time, the changes in habitat complexity, connectivity and recruitment.

The recovery of coral communities is defined as both the recovery of coral cover and the reassembly of coral community to pre-impact. One of the most alarming trajectories of coral reef recovery is the shift from a coral dominated reef to a macroalgal-dominated reef. Once established, algae will outcompete coral for space (McCook et al. 2001) and can prevent corals from recruiting and returning to a coral dominating reef, compromising the ecosystem functions of the reef (Mumby 2009). This transition from coral to macroalgae (or other organisms such as sponges) has been described as a phase shift (Done 1992; Hughes 1994; McCook et al. 2001; McManus and Polsenberg 2004; Hughes et al. 2007; Bruno et al. 2009; Norström et al. 2009) and has been linked to a reduction in herbivory and increase in nutrient availability (Mumby and Steneck 2008).

The diversity of species of coral present on a reef dictates the structure of fish and invertebrate communities and plays an important role in ecosystem function (Chabanet et al. 1997; Feary et al. 2007; Olds et al. 2012). Diversity can also play a role in the overall resilience of the reef. Studies have shown that coral species have different thermal threshold with some being more resistant to bleaching than others (Marshall and Baird 2000; Golbuu et al. 2007; Brandt 2009), for example *Acropora* is usually less tolerant than *Porites*. These generalizations are also often linked to the morphological characteristic of the colony where branching forms are more susceptible than massive forms (Loya et al. 2001). Reefs that loose their biodiversity or recover from only

susceptible corals will become less resilient to future stresses. However, to date little is known about the succession of coral species after a major disturbance.

Palau International Coral Reef Center (PICRC) has been monitoring data of coral reefs in Palau since 2001. In 1998, Palau's reef suffered extensive bleaching mortality that was widespread across depth, sites, habitats and taxa (Bruno et al. 2001). Approximately one-third of the coral died, with areas suffering as high as 70% mortality (Bruno et al. 2001). Since then, the reef has recovered as observed by the long term monitoring carried out by the team of researchers from PICRC (Golbuu et al. 2007; Victor et al. 2009). I use PICRC monitoring data from 2001 to 2009 and a survey carried out by The Nature Conservancy (TNC) in 2012 to describe the recovery pattern of the reef of Palau. In particular I look at the interaction between algae and coral cover and the succession of corals after disturbances. I also look at factors that influence the patterns of recovery, including the rate of coral recovery and the total cover in 2012.

Methods

Site description

The Republic of Palau is a reef complex located on the westernmost end of Micronesia. It supports over 500 km^2 of coral reef with a population of approximately 21,000. In 1998 the corals of Palau suffered high mortality from a bleaching event and a comparison of coral reefs between 1992 (Maragos and Cook 1995) and 2001 (Bruno et al. 2001) showed a decline in coral cover. In response to the loss of corals, the government of Palau increased their efforts to protect the reefs and by 2007 there were 31 MPAs protecting over 40% of Palau's nearshore marine area (Golbuu et al. 2005).

Current threats and future impacts

While Palau's reefs have been generally well managed through traditional and national laws (Golbuu et al. 2005), several studies have shown that some reefs areas, in particular those in bays, are impacted by terrestrial run-off and sedimentation (Victor et al. 2005; Golbuu 2011b; Golbuu et al. 2011). Although less prevalent than on other Pacific islands, fishing remains a source of stress for the reefs and monitoring reports of the marine

protected areas in Palau show a reserve effect where biomass is higher inside the reserve than outside (Friedlander et al. 2014; Koshiba et al. 2014).

In addition to existing threats, several studies have shown that Micronesia, and in particular Palau, is located in a region that will be one of the first to be exposed to thermal anomalies (Donner et al. 2005; Donner 2009; van Hooidonk et al. 2013). van Hooidonk et al. (2013) state that in the best case climate modeling scenario (i.e., RPC6.0) where there will be a reduction and stabilization of CO_2 emissions, bleaching events will occur annually across reefs worldwide by 2078 while Micronesia will surpass thermal bleaching thresholds annually as early as 2040.

Field Work and data processing

Monitoring data 2001-2009

Monitoring data was collected by the Palau International Coral Reef Center (PICRC) between 2001 and 2009. A detailed description of the field work, collection and post collection processing is described in Golbuu et al. (2007). Briefly, PICRC started a nationwide coral reef monitoring program in 2001 that was conducted more or less every other year, providing data from 2001 and 2009 (Table 5.1). The group collected data from thirteen permanent study sites with nine sites on outer reefs that are wave-exposed; four on the east coast and five on the west coast. A further two sites were located on patch reefs, and two within sheltered bays (Fig. 5.1). PICRC added seven more sites between 2003 and 2005, five of which were exposed and two in bays (Fig. 5.1). Five 50m transects were deployed at two depths, 3 and 10m, following depth contour and coral communities were recorded over a 50x1m belt transect using a digital video.

To obtain coral cover information, images were systematically extracted to provide 40 images per $50m^2$ belt transect. For each frame, five random points were selected and assigned benthic categories. Data was transformed into 100% cover.

Two final datasets were created: one with the PICRC data from 2001 to 2009 and one with the PICRC data and the 2012 TNC benthic surveys. To add the TNC data, benthic categories were pooled and simplified (Appendix 14). Only six categories were retained:

live coral cover, algae, rubble, hard substrate (with or without crustose coralline algae), sand and other (including, but not restricted to, mushroom corals, sponges and ascidians). Live coral cover was additionally divided into resilient, intermediate and susceptible classes depending on taxonomic family or growth forms (Appendix 14) based on the previous work of Loya et al. (2001), McClanahan et al. (2004), Marshall & Schuttenberg (2006) and Woesik et al. (2012). Massive and submassive are categorized as resistant, foliose and encrusting as intermediate and branching, bushy and table as vulnerable Marshall & Schuttenberg (2006). The monitoring dataset from 2001 to 2012 was then organized by year, site, depth, transect, benthic category, % cover, bleaching susceptibility and exposure. Exposure was defined by geographic location which reflects the exposure of the sites to seasonal wave action and divided the reef into exposed north, northeast, northwest, southeast, southwest and south and sites protected in bays (Table 5.1). The dataset with only the PICRC data retained information on coral genera and was organized by year, site, depth, transect, benthic coral genera and % cover.

Data analysis

Monitoring data 2001-2012

To understand general trends in the trajectory of the benthic substrate, I first plotted in R (v.3.2.3, R Core Team (2008), ggplot2 version 2.1.0) the benthic categories against year by site and depth (Fig. 5.2). I plotted for each depths and at each transect the proportion of macroalgae, live coral and bare substrate (with or without coralline crustose algae) over time using ternary diagrams in R (ggtren v2.1.4, Hamilton (2016)) (Fig. 5.3a,b) to detect phase shifts. A ternary plot, or triangle plot, is a barycentric plot on three variables, which sum to a constant. Graphically it illustrates the ratios of the three variables as positions in an equilateral triangle.

To characterize the recovery of the coral community after the initial loss of coral cover from the 1998 bleaching event, I plotted % live coral cover over time in R (ggplot2 v 2.1.0) by sites and depth and fitted linear regressions to the data (Fig. 5.4). Although not all sites exhibit a linear relationship between % coral cover and years, enough sites show a random residual distribution in order to use the slope of the regression as a general indicator of the average amount of change over time. To rank the slope of coral cover

over time and the average coral cover over all years by site and depth, I plotted the random factors years, depth nested by site of a linear mixed effect model with no fixed factors in R (lme4 v. 1.1-12, effects v 3.1-1, Fig. 5a, b). To see if the slope and average coral cover was significantly different by depth I first transform the percentage cover data with the logit function in R (car v. 2.1-2) to normalize it. I ran a full mixed model with year, depth, exposure and resilience score as fixed factors and year and depth nested within sites as random factors. I compared it to the same model without depth in the fixed factors and ran a likelihood ratio test (anova).

Coral reef communities are known to vary with exposure to wave energy (Done 1982; Hughes et al. 2012; Graham et al. 2014) and Roff et al. (2015) also indicate a difference in recovery of corals in Palau after a 2012 typhoon. To tease out the effect of exposure on the recovery and average live coral cover, I fit the transformed data using R (lme4 v. 1.1-12) in the full linear mixed model and I test the significance of the effect of exposure with a likelihood ration test by removing exposure from the fixed effects. I use R (ggplot2 v. 2.1.0) to plot live coral cover over time by exposure classes and by depth (Appendix 15).

Community succession was analyzed using two different indicators: biodiversity, calculated as the number of different genera on the reef and bleaching susceptibility. Using R (effects v 3.1-1) I plotted the random effects of a linear mixed effect model with % live coral cover against the random variables year, site and bleaching susceptibility categories for 3 and 10m (Appendix 15). I use R (ggplot2 v. 2.1.0) to plot live coral cover over time by exposure classes and by depth (Appendix 15). For each transect, the barycenter of vulnerable, intermediate and susceptible coral cover was plotted in R (ggtren v2.1.4, Hamilton (2016)) in ternary diagrams by depth (Fig. 5.6a, b).
I plotted in R (ggplot2 v. 2.1.0) average biodiversity by depth and site over the years (Fig. 5.7).

Results

Monitoring data 2001-2012

Live coral and hard substrate cover show the most changes over time (Fig. 5.2). Algae cover shows very little changes at 3m except for a loss of cover at Ngelukes. At 10m Ngaremlengui patch and Ngerchong show an increase in algae cover. The ternary plots show a progression from transects with proportionally low coral and algae cover against bare substrate cover to a reef with proportionally high coral cover against bare cover and algae for both depths (Fig. 5.3a, b). At 10m, however, there are several transects where algae cover proportionally increases (Fig. 5.3b). Ngaremlengui patch reef, however, is the only site with higher algal cover than coral cover. This site is one of two sites on a patch reef inside the lagoon, as opposed to the exposed barrier reef.

The trajectories of live coral cover over time can be divided into three scenarios (Fig. 5.4): live coral cover measured during the first survey (2001-2002) is low (=<25%) but increases over the next 11 years; live coral cover is moderately high (between 25 and 50%) in 2001 and slowly increases over the years and live coral cover is high (>50%) in 2001 and either stays stable or slightly decreases. I find an effect of depth on coral cover and on the slope of coral cover over time ($AIC_{\text{Full Model}} = 2098.7$, $AIC_{\text{without depth}} = 2101.6$, $p=0.018$).

The sites with the largest positive slopes for live coral cover are located on the west barrier reef at 3m (Fig. 5.3a). The sites with highest average percent live coral cover over all years are the sites in bays: all three Nikko sites and Taoch sites both at 3 and 10m (Fig. 5.3b). Exposure has a significant effect on live coral cover ($AIC_{\text{Full Model}} = 2098.7$, $AIC_{\text{withoutExposure}} = 2123.4$, $p<0.01$). The largest difference measured is for sites located in the bays (Estimate = 2, Table 5.2) which have the highest average coral cover but a very small slope. The greatest increase in coral cover takes place in the northwest sites at 3m and the southwest sites at 10m (Appendix 14).

Biodiversity increases at all sites for both depths, even in bays where there was little loss of coral cover. Bays have the highest overall biodiversity. The succession of corals by

bleaching susceptibility differs between sites located in the bays and on exposed reefs. In bays, resistant forms have the highest average coral cover with a negative slope of coral cover over the years, whereas the opposite is true for exposed reefs (Appendix 15). The ternary plots show that in 2001 most of the reef had lost their susceptible corals with a majority of resistant and intermediate forms. The proportion of susceptible and resistant corals increases with years. For both depths, the 2012 values seem to be outliers (Fig. 5.6).

Discussion

Recovery patterns of coral cover from 2001- 2012

Fourteen years after a significant loss of corals from the 1998 coral bleaching event, the reefs of Palau have recovered to their pre-bleaching coral cover of over 50% for exposed reefs, and has stayed at 30% for fringing reefs in bays (Maragos and Cook 1995; Golbuu et al. 2007). Exposure was a significant factor to determine difference in the rate of recovery and total coral cover. Three recovery scenarios correlate with reef exposure to wave actions: sites on fringing reefs in bays (Nikko and Taoch) did not suffer much loss of coral cover and coral cover stays constant or slightly decreases. Sites in the northeast (Ngaraard and Melekeok) initially did not lose as much coral cover as western reefs but are recovering more slowly (Fig. 5.4).

The difference in recovery patterns between sheltered bays and exposed bay were previously described by Golbuu et al. (2007) and Woesik et al. (2012). Similarly to Woesik et al. (2012) who attributed the differences of mortality to coral communities in bays that were less susceptible to bleaching, I found that the reefs in the bays had more resilient forms of corals. Woesik et al. (2012) also attributed higher resistance of corals in bays to acclimatization to high surface temperatures and high concentration of particles in the bays that reduce irradiance and protect corals from bleaching which may explain the difference in the initial mortality I observed. It should be noted, however, that the biodiversity of the reefs increased in the bays indicating that some of the more cryptic coral genera in the bays were affected by the bleaching event.

It is possible that the corals that survived in the bays helped the fast recovery of the reef of Palau. The difference in species composition in coral communities in bays and on exposed reefs, however, reduces the possibility of connectivity, with many of the species occurring specifically in one habitat (Golbuu et al. 2007).

Differences between northern and southern barrier reefs were previously described in Golbuu et al. (2007, 2012), Victor et al. (2009) who explained the lower coral cover and slower recovery on the northern reef by lower larval retention and recruitment. This correlates to findings in chapter 3 of lower self-seeding on the northern reef than the southern reef.

The interaction between algae, coral and bare substrate cover shows that when the reefs of Palau lost coral cover, very little algae colonized the empty space. Instead, the equilibrium moved from bare substrate to live coral. This coincides with the observations by Bruno et al. (2009) in the Indo-Pacific where very few coral communities, even after impact, show signs of algal growth and or a switch to an algal dominated state. At 10m, however, there is a trend toward increasing algal cover. The difference in algal cover between 3 and 10m could be due to a difference in herbivory between these two depths (Fox and Bellwood 2007; Hoey and Bellwood 2008) with fewer herbivores grazing at 10m to maintain low algal cover (reviewed in McCook, Jompa & Diaz-Pulido(2001); McManus & Polsenberg (2004); Hixon (2015)). Although Palau is an area that is recognized as well managed, reports from PICRC monitoring show effects of reserve on fish biomass (Koshiba et al. 2014), which indicate fishing pressure on fish communities.

The difference in algal cover between the two depths could have significant implications for conservation. It has been suggested that deeper coral reefs may act as refugia to shallower reefs that may be more impacted by thermal stresses (Glynn 1996; Riegl and Piller 2003; Smith et al. 2014). It is therefore important to focus on the coral-algae-herbivore interactions at deeper sites in Palau, and conservation efforts include the protection of reef slopes, extending beyond the reef crest.

Several studies (Golbuu et al. 2007; Victor et al. 2009; Cros et al. 2016a) have shown that corals in Palau have recovered from local sources of larvae from colonies that survived the 1998 bleaching event. Either those colonies were in locations that acted as refugia, such as shaded or cooled areas, or the colonies themselves were resistant to bleaching. Although both scenarios can take place simultaneously, I show that the cover of resistant corals increased between 2001 and 2010, indicating that resistant corals survived and recolonized the reef. Susceptible corals recovered about 6 years after the bleaching event. The recovery of reef by corals that were resistant to a bleaching event could increase the resilience of the reef to future thermal stress, as has been observed in Kaneohe Bay (Bahr et al. 2015).

The 2012 outliers can be explained by the difference in method to assess coral resilience class. From 2001 to 2010, benthic susceptibility was assessed on genera or species whereas in 2012, benthic susceptibility was assessed on growth forms only and could result in a different classification of corals. Although there is little information on the coral composition of the reefs of Palau prior to the 1998 bleaching event (Maragos and Cook 1995; Bruno et al. 2001), I recommend an assessment of the species of corals that suffered the most mortality and create an informative classification of bleaching susceptibility specific to Palau.

Management recommendations

The reefs of Palau recovered quickly since 1998 and coral cover has continued to increase at most sites around Palau indicating a resilient ecosystem. Nonetheless, there are indications that reefs at 10m may be impacted by fishing pressure and removal of herbivorous species. Reefs in the bays are also indicating small decreases in coral cover, which could be an indication of increased direct anthropogenic impact such as pollution, sedimentation as signaled by previous studies (Golbuu et al. 2005, 2011; Victor et al. 2005) although it could also be due to the impacts of the 2010 bleaching event (Woesik et al. 2012). Since both reefs at deeper depths and within the bays of the lagoon of Palau have been identified as potential areas of refugia for future bleaching events (Woesik et al. 2012), I recommend increasing protection of both through expending no take areas to

include reef slopes and by better managing the watershed to reduce sedimentation as much as possible in the bays and lagoons.

Tables

Table 5.1. PICRC permanent monitoring site and TNC resilience survey sites. GPS coordinates and years surveyed.

Site	Longitude	Latitude	PICRC						TNC	Exposure
Airai	134.555	7.327	2001	2012	2003	2005	2006	2007	2009	SE
Kayangel	134.686	8.0427	2001	2012	2003	2005	2006	2007	2009	N
Melekeok	134.636	7.520	2001	2012	2003	2005	2006	2007	2009	NE
Ngaraard	134.649	7.586		2012	2003	2005	2006	2007	2009	NE
Ngaremlengui barrier	134.468	7.556	2001	2012	2003	2005	2006	2007	2009	NW
Ngaremlengui patch	134.488	7.554	2001	2012	2003	2005	2006	2007	2009	NW
Ngelukes	134.607	7.418		2012	2003	2005	2006	2007	2009	SE
Ngemelis	134.239	7.114	2001	2012	2003	2005			2009	SW
Ngerchelong patch	134.572	7.818	2001	2012	2003	2005	2006	2007	2009	NW
Ngerchong	134.365	7.110	2001	2012	2003	2005			2009	SE
Ngerdiluches	134.345	7.418	2001	2012	2003	2005	2006	2007	2009	SW
Ngetngod	134.619	7.362		2012		2005	2006	2007	2009	SE
Nikko 2	134.499	7.489				2005	2006	2007	2009	SB
Nikko 3	134.499	7.329		2012		2005	2006	2007	2009	SB
Nikko Bay	134.494	7.326	2001	2012	2003	2005	2006	2007	2009	SB
Peleliu	134.217	7.006	2001		2003	2005	2006	2007	2009	S
Tsais Reef	134.231	7.306	2001	2012	2003	2005	2006	2007	2009	SW
Taoch 1	134.427	7.276	2001	2012	2003	2005	2006	2007	2009	SB
Taoch 2	134.407	7.279		2012		2005	2006	2007	2009	SB

Table 5.2. Random and Fixed effects of a mixed linear model.

Model: lmer(Cover ~ Year + Depth + Exposure + Year:Depth + Year:Exposure + Depth:Exposure + (Yearcent | Site:Depth))

Random effects:

Groups	Name	Variance	Std.Dev.	Corr
Site:Depth	(Intercept)	0.30	0.55	
	Year	0.00	0.05	-0.25
Residual		0.32	0.57	

Number of obs: 1101, groups: Site:Depth, 38

Fixed effects:

	Estimate	Std. Error	t value
Depth3(Intercept)	-1.65	0.35	-4.69
Year	0.21	0.05	4.65
Depth10	1.18	0.64	1.83
ExposureNE	1.14	0.34	3.38
ExposureNW	0.14	0.41	0.34
ExposureS	0.19	0.66	0.29
ExposureSB	2.09	0.43	4.85
ExposureSE	0.16	0.38	0.43
ExposureSW	0.33	0.47	0.71
Year:Depth10	-0.04	0.02	-2.01
Year:ExposureNE	-0.04	0.06	-0.69
Year:ExposureNW	0.05	0.05	1.04
Year:ExposureS	-0.10	0.07	-1.52
Year:ExposureSB	-0.18	0.05	-3.58
Year:ExposureSE	0.00	0.05	-0.02
Year:ExposureSW	0.00	0.05	0.02
Depth10:ExposureNE	-1.56	0.71	-2.20
Depth10:ExposureNW	-1.10	0.74	-1.48
Depth10:ExposureS	-0.21	1.01	-0.21
Depth10:ExposureSB	-1.77	0.73	-2.42
Depth10:ExposureSE	-1.37	0.70	-1.97
Depth10:ExposureSW	-0.08	0.78	-0.10

Figures

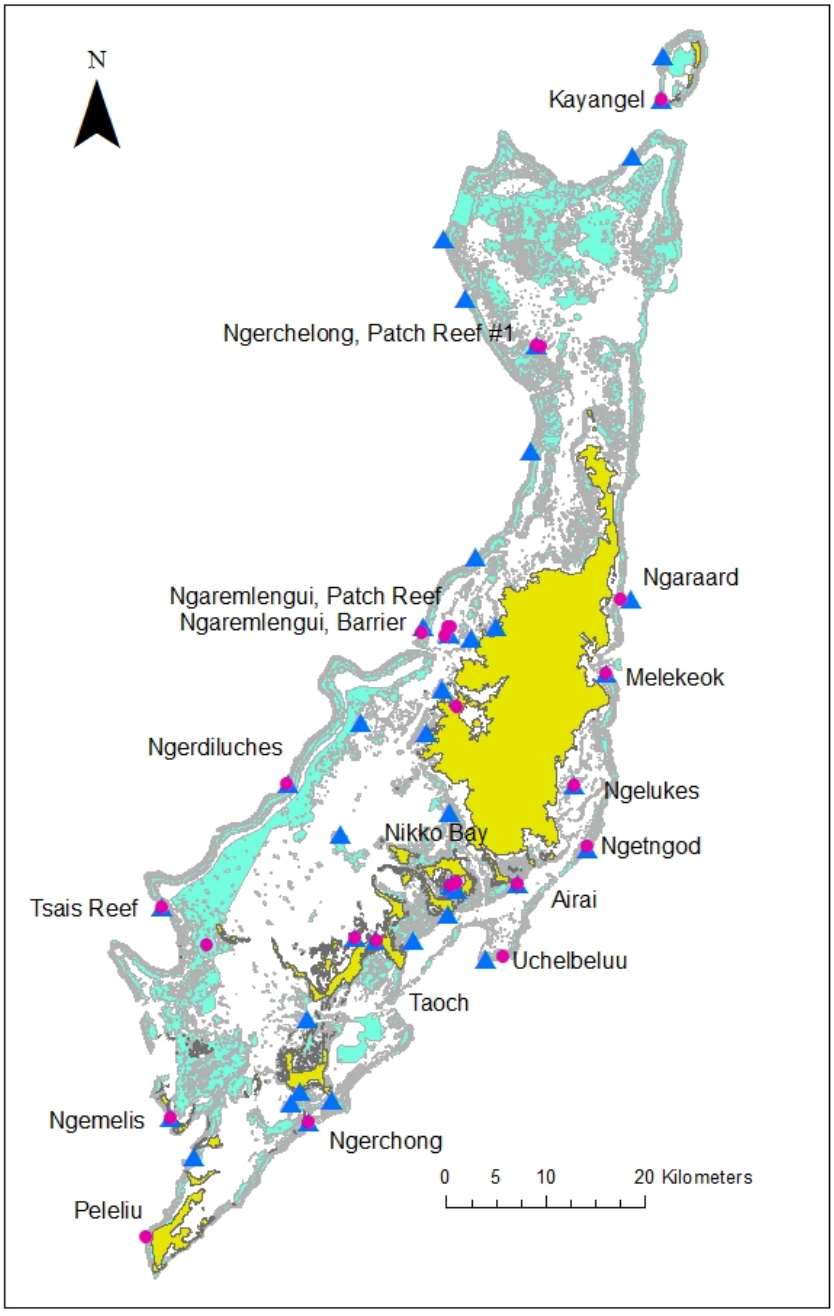

Figure 5.1. Map of Palau. In pink circular shapes are the PICRC permanent monitoring sites and blue triangle shapes are TNC resilience sites.

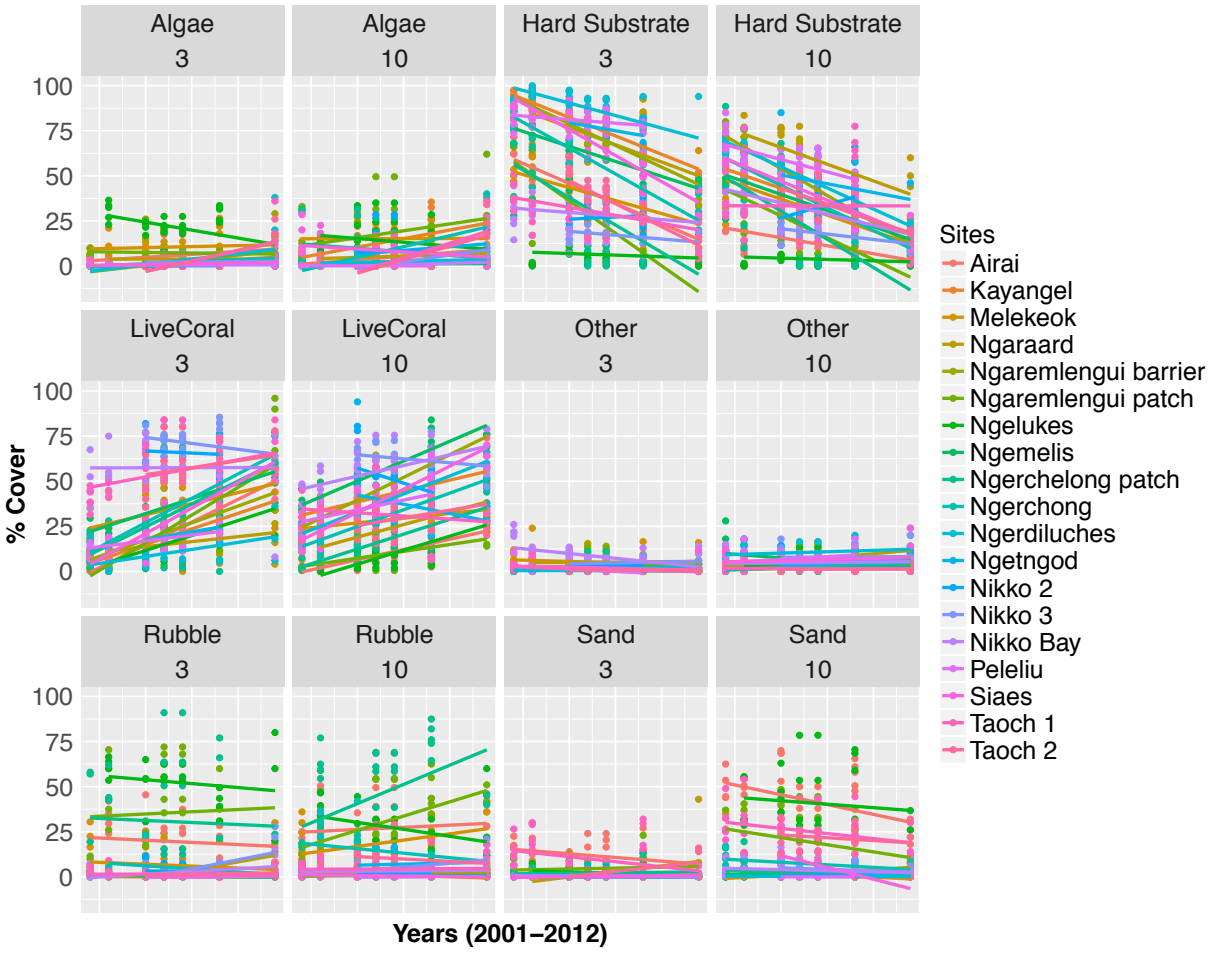

Figure 5.2. Multiple plots of percent cover of six benthic categories over seven years for 19 PICRC permanent monitoring sites at 3 and 10m. Each site is represented by a different colour.

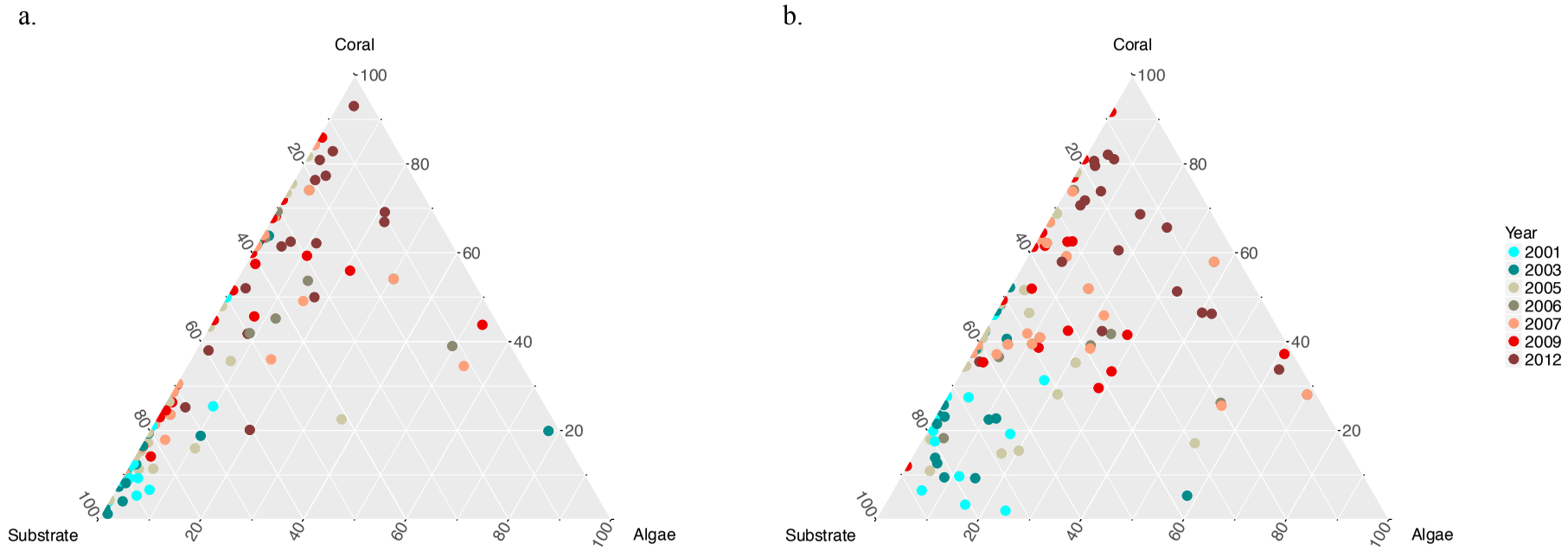

Figure 5.3. Ternary plot of barycentric values for 19 sites for three benthic cover, live coral, algae and bare substrate over seven year at 3m (a) and at 10m (b). Each year is represented by a different colour, with warmer colours representing recent years.

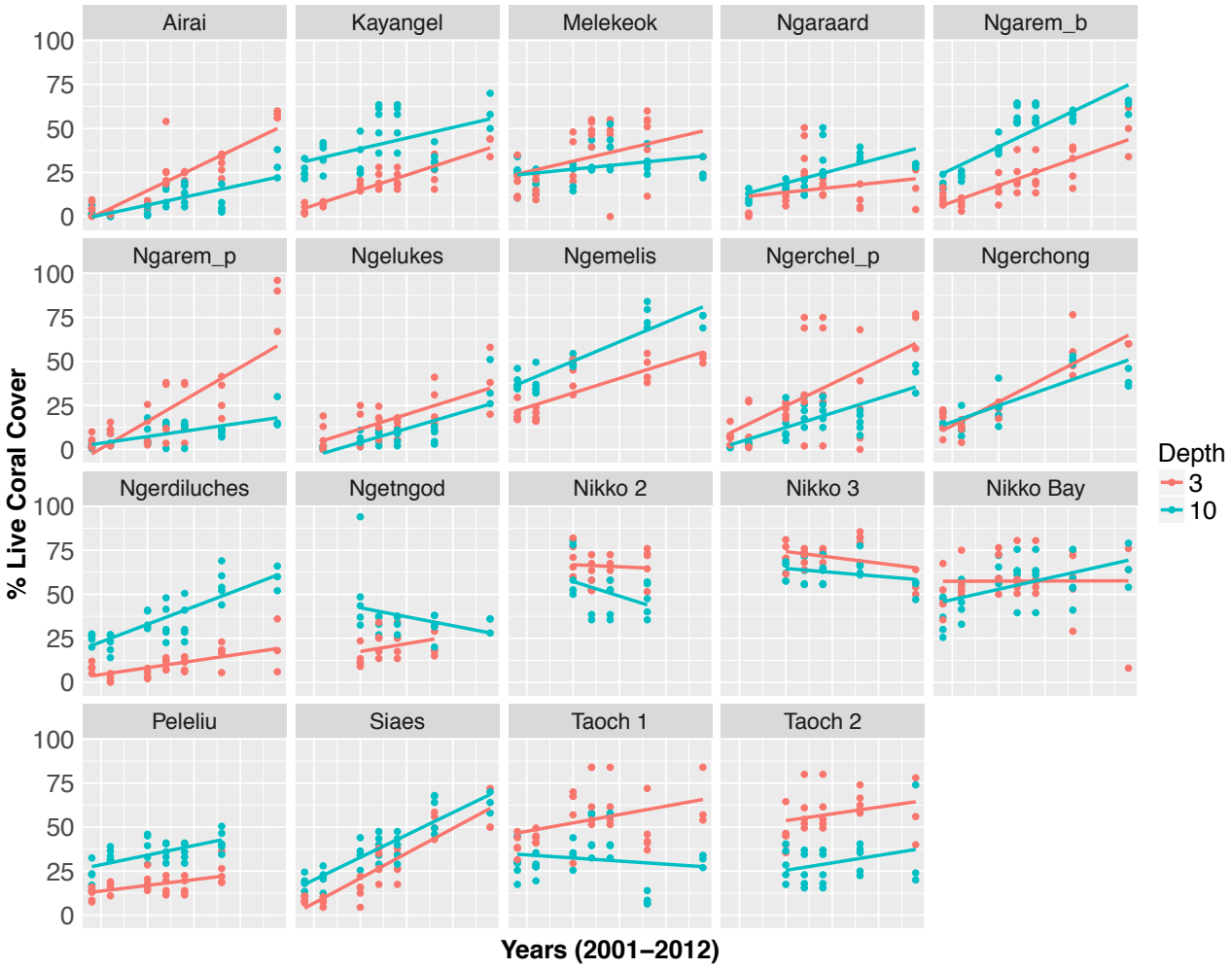

Figure 5.4. Multiple plots of percent live coral cover over seven years for 19 PICRC permanent monitoring sites at 3 and 10m. In pink the best fit for transects at 3m and in turquoise, best fit for transects at 10m.

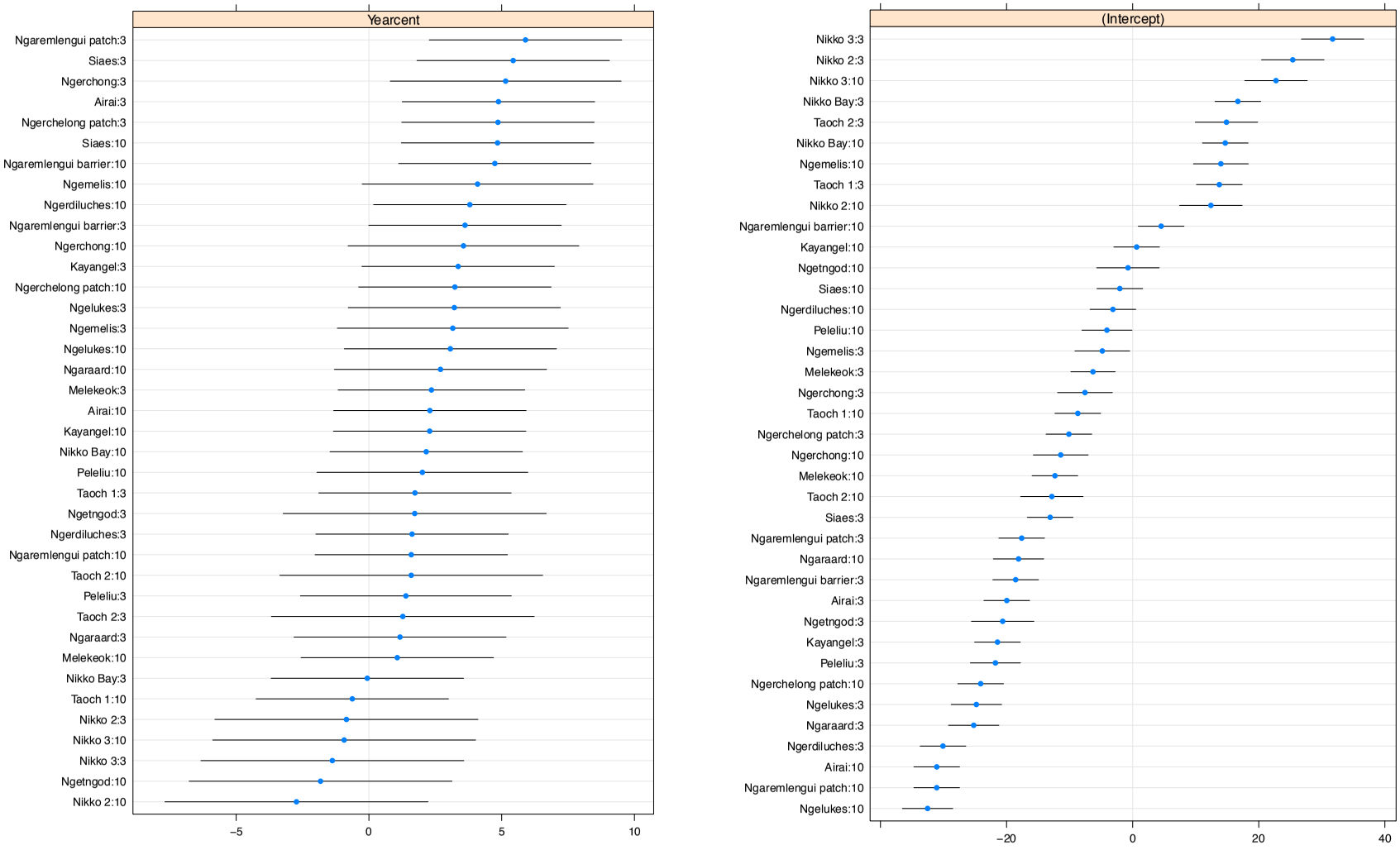

Figure 5.5. Rank of average percent live coral cover by site and depth (a) and the rank of slopes of linear regression of percent live coral cover over time by site and depth (b).

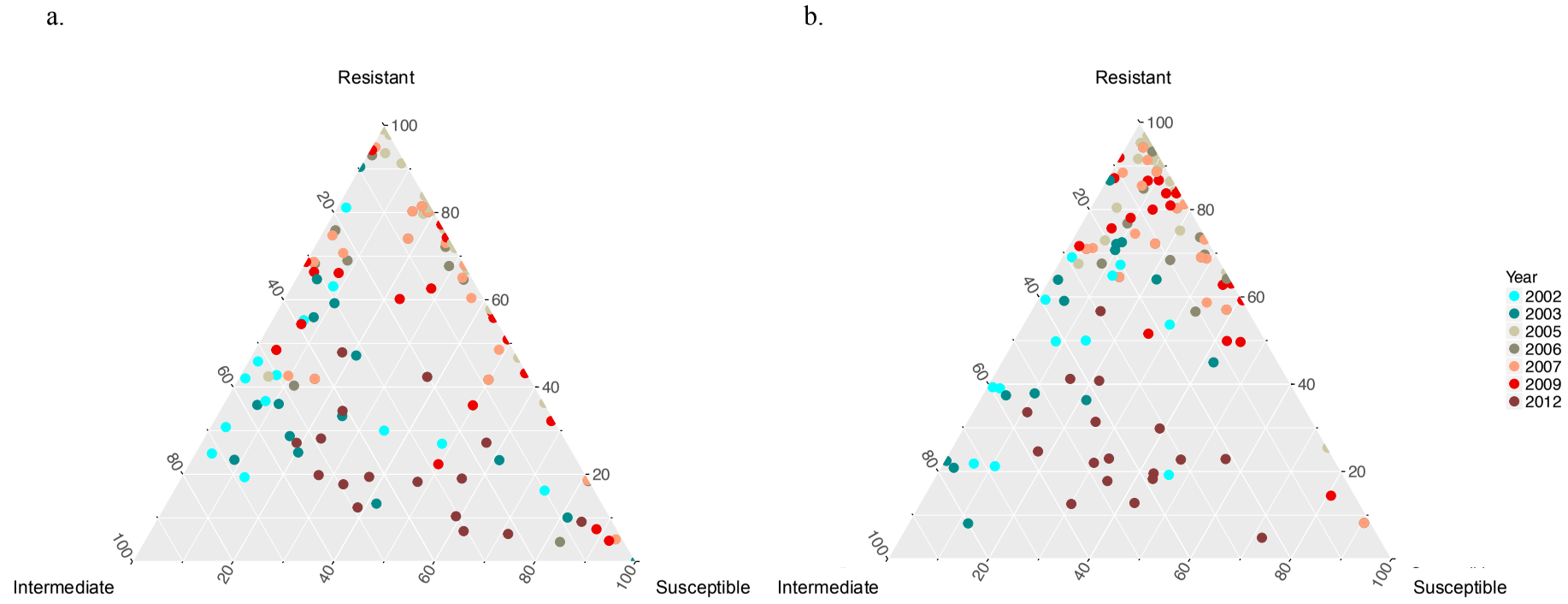

Figure 5.6. Ternary plot of barycentric values of live coral cover for 19 sites for three bleaching susceptibility, resistant, intermediate and resilience over seven year at 3m (a) and at 10m (b). Each year is represented by a different colour, with warmer colours representing more recent years.

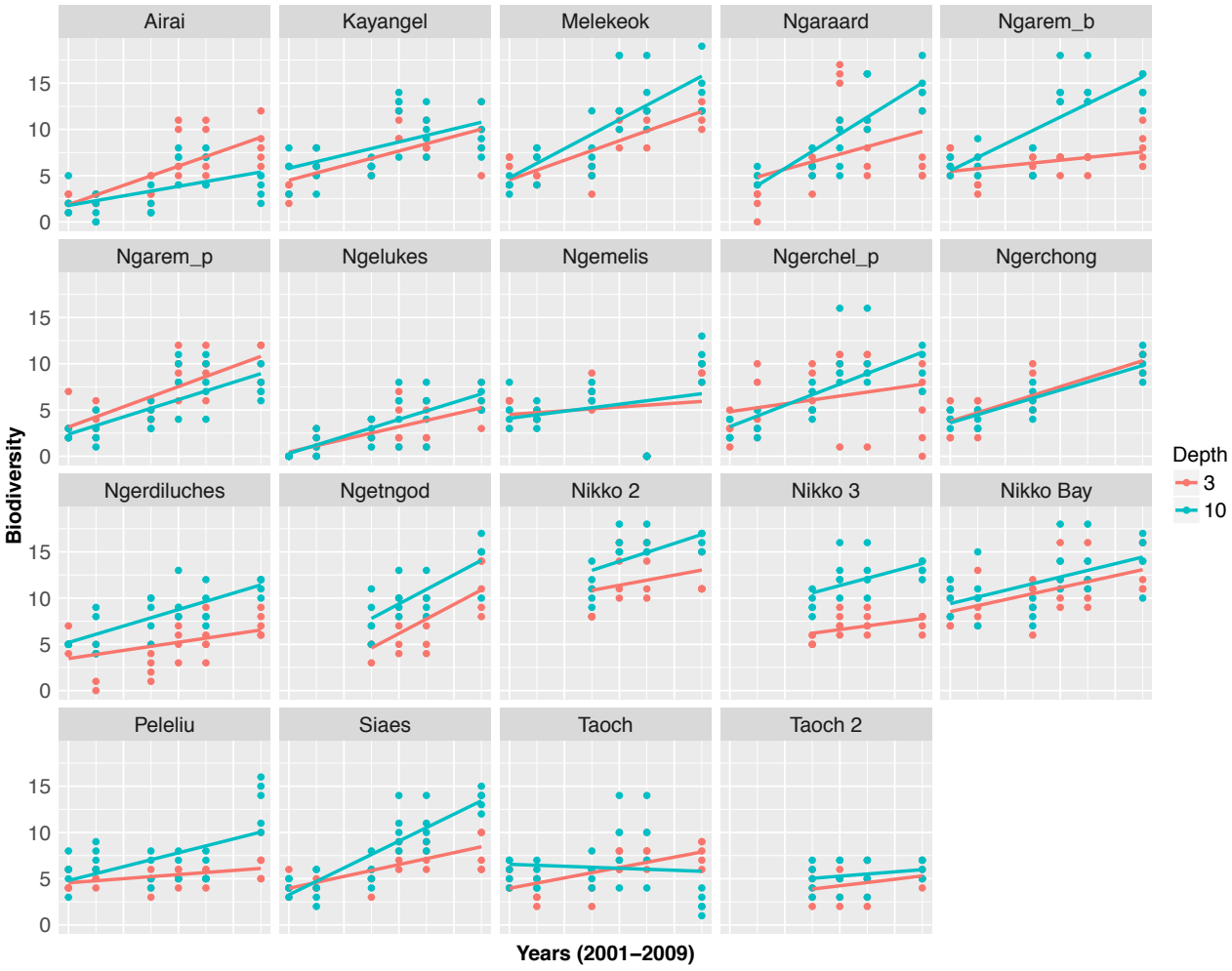

Figure 5.7. Multiple plots of biodiversity over eight years for 19 PICRC permanent monitoring sites at 3 and 10m. In pink the best fit for transects at 3m and in turquoise, best fit for transects at 10m.

CHAPTER VI. SUMMARY AND CONCLUSION

Summary

Understanding connectivity and the role it plays in enabling the recovery of coral reefs remains challenging. Advances in molecular ecology and population genetics are providing new tools to answer some fundamental questions on the role of larval dispersal on demographic processes and population structure. By applying those tools in Palau, we are able to better understand the recovery of its coral reef after the 1998 bleaching event.

The coral reefs of Palau recovered quickly both in terms of coral cover and biodiversity. Recovery, however, was not uniform. Corals in sheltered bays were less affected than corals on exposed reefs, and corals in the northern barrier reef recovered more slowly than those on the southern barrier reefs. Many factors can explain these differences, such as the composition of coral communities, bleaching susceptibility of coral species, larval retention or water circulation, which are discussed in Chapter V.

Understanding where larvae came from for new coral recruit and reef recovery has important consequences for management strategies. This question is addressed in Chapters II, III and IV using population genetics and observing the recovery mechanisms for a single species, *Acropora hyacinthus*. What emerged from this study is that Palau did not recover from long distance dispersal from Yap or Ngulu (Chapter II), or from several refugia around Palau (Chapter III). Rather, bleaching did not impact all coral colonies along the reefs and those that survived re-seeded the reefs (Chapter III and IV). This re-seeding took place at very short scale (self-seeding), creating genetic neighborhoods that, with time, will eventually grow and mix with each other. After fourteen years, the reef has not completely recovered and still forms mosaics of genetically different coral populations (Chapter IV).

This research adds to the body of literature that shows that population connectivity and larval dispersal in the marine realm is more restricted than predicted. *Acropora hyacinthus* is a broadcast spawner with a larvae that is capable of surviving in the water column for weeks and has the potential to disperse over several kilometers. Realized dispersal, however, is restricted to hundreds of meters. This doesn't exclude the

possibility of long distance dispersal but the majority of larvae of *A. hyacinthus* travel short distances, staying close to home and that a short dispersal of a few hundred meters is shaping population structure of *A. hyacinthus*.

Management recommendations

As thermal stresses are predicted to increase in frequency, it is important for managers to understand which processes help coral reefs to recover and maintain their resilience. One approach is to use population genetics to study connectivity and population structure. Although it can be an expensive and lengthy process, it can highlight key processes such as larval dispersal. Here, I was able to make several management recommendations based on the genetic structure of the population of *A. hyacinthus* in addition to ecological observations.

The lack of evidence of the role that played long distance dispersal in structuring the reef leads to the recommendation of increasing conservation effort in Palau and not at a regional level. Although regional networks of MPAs may provide additional resilience to the system over a number of years, it will not help Palau's reefs recover at the same rate as it did after the 1998 bleaching event.

The difference in recovery between the corals in the sheltered bays and exposed barrier reefs indicate that these are very different reefs systems that are exposed to different stresses. I recommend treating them as two independent management units, each with their own management strategies. Due to the difference in coral communities it is unlikely that there is much connectivity between sheltered and exposed reefs, however this still needs to be demonstrated.

A similar approach should be taken for the barrier reef, dividing it by wave exposure. I recommend in particular developing a management plan for the southern reef because coral populations showed evidence of higher self-seeding than anywhere else on the reef making them less resilient to bleaching.

Corals survived all along the barrier reef, inside and outside of MPAs and recruitment took place at very short scale with reefs recovering outside of MPAs. A key component of recovery is for coral larvae to have available space to settle and recruit. To ensure that this is possible anywhere on the reef, priority should be given to managing reefs outside MPAs. I recommend managing fisheries outside of MPAs to keep algae levels low and increase reef resilience. Furthermore, managing reefs outside of MPAs will allow to capture more of the mosaic of genetic diversity displayed by the reefs of Palau which may be impossible to protect through MPAs.

Next Steps

The question of the existence of areas that can offer shelter to corals during bleaching events still remains unanswered. *Acropora hyacinthus* is a coral that, in Palau, occurs on exposed barrier reefs, with very few colonies found on patch reefs and is absent in sheltered bays. It grows at depths ranging from 1 to 15m, allowing it to find refugia in deeper waters, explaining the survival of colonies along the reef even when most shallow colonies are dead. Other species of coral occurring within and outside sheltered bays may also find refugia in the bays. Both depth and habitat refugia can be tested by understanding connectivity among populations using a similar population genetics approach as the one used in this study.

The use of population genetic tools to inform management on connectivity is currently a lengthy and expensive process. This is in part due to the high number of samples required and lengthy laboratory steps. With the advances of technology, however, this work is becoming increasingly more accessible and affordable. Developing methods to optimize the sample size and therefore cost and time would allow to duplicate this approach to other reefs to inform management developing resilient MPA networks.

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APPENDICES

Appendix 1. List of 18 primers amplified, modified from Wang, Zhang & Matz (2009). "Primer #": primer number as labeled in our dataset; "Locus": name of the locus as per Wang, Zhang & Matz (2009); "Repeat motif": short tandem repeats; "Size": size range of the microsatellite found by the authors; "Allele #": number of allele found by the authors; "N": number of individuals sequenced, "H_O": observed heterozygosity; "H_E": expected heterozygosity; "p": p value from exact tests of HWE.

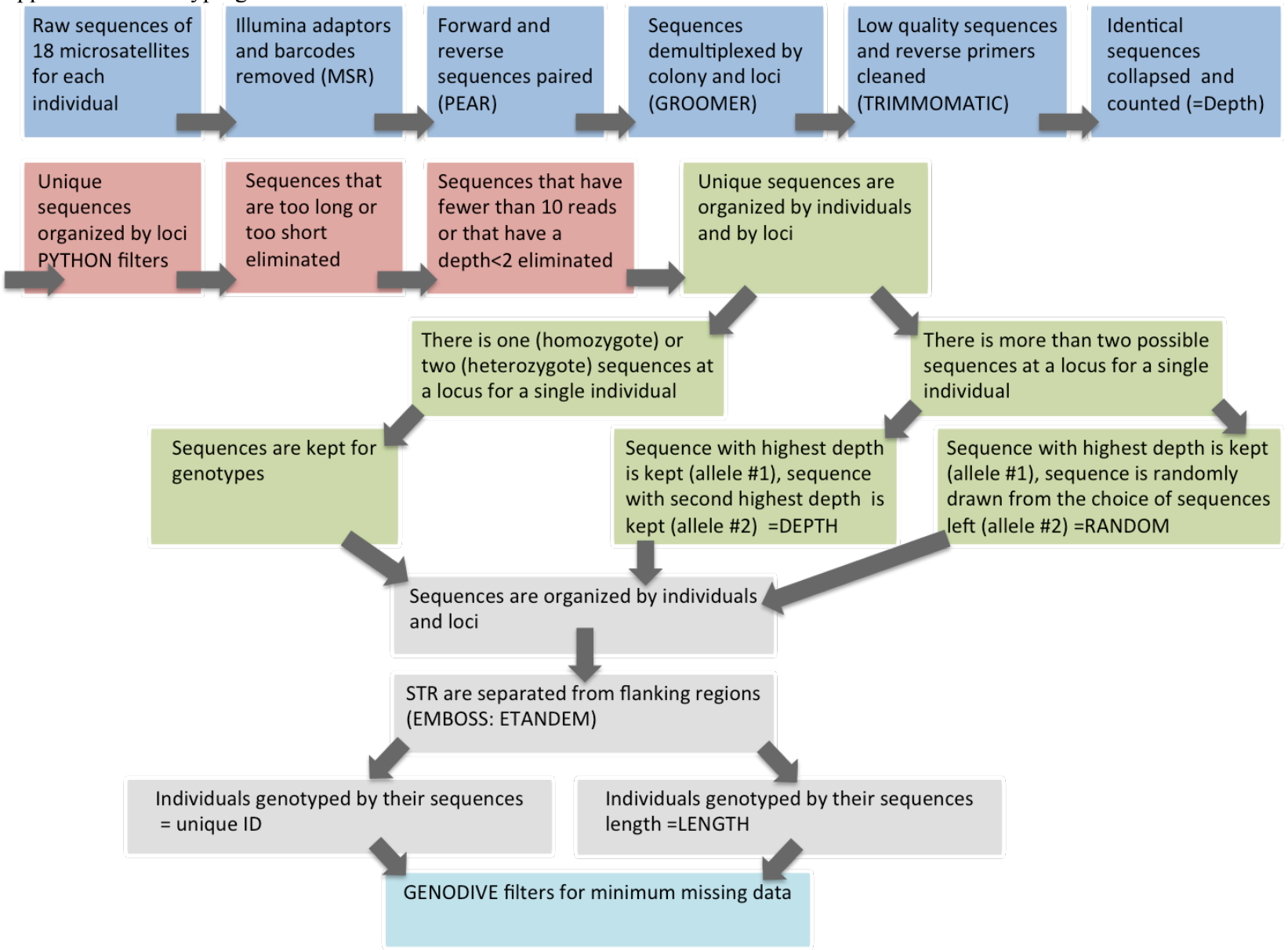
Primer #	Locus	Repeat motif	Primer sequence (5'-3')	Size (bp)	Allele #	N	H_O	H_E	p	Accession #
1	EST007	(TTTC)5	F: TGCAATGGTTCTGTTGCAGTCA R: GATCTCTTTACCGATTTACAGCA	99-107	3	24	0.38	0.47	0.2025	DY587595
2	EST014	(TCT)13	F: AGCCGAAGAGGGGACAGAGT R: AGCCGAAGAGGGGACAGACT	143-173	10	24	0.92	0.88	0.6926	DY586774
3	EST016	(AAC)7	F: CTATCTGTGTATGATCAGGACTA R: TCCATCTGTTGTGGAAACTGGT	97-122	7	24	0.67	0.69	0.1383	DY586537
4	EST032	(TTA)21	F: AGGCACAAGAAAGTGGAAAACAA R: TGAAGGGATGTGAAGCATGGT	138-187	15	24	0.96	0.94	0.7274	DY585386
5	EST062	(GAT)9	F: CGAGTTAGTCTGTTAAGATGGT R: CTCTAAGTCCGATCTTCTTCCA	110-126	5	24	0.67	0.71	0.1437	DY58448
6	EST097	(TGA)7	F: TGACAACGACATCAATCATGGT R: ACAGCAGGAGCTGTCAGCACT	123-135	5	24	0.71	0.69	0.0923	DY583334
7	EST098	(TG)12	F: ACAAATTGCGCTCAAGTTGATG R: ACGGCTGCGAAGGAGTCTAGT	98-118	8	24	0.58	0.68	0.0362	DY583314
8	EST181	(ATG)10	F: TGATTGCTGAGAAAGCTAGAGAT R: GCCTCACCTTGCCTTGTACA	145-157	2	24	0.25	0.22	1	DY580714
9	EST196	(TAA)9	F: GTGTTGGCTATCTCATGTATAGT R: ACAACACATCATCAACAACAGCA	117-145	9	24	0.79	0.85	0.0926	DY580091
10	EST254	(CA)10	F:GGTGACCAATCAGAGTCTTGA R: TACACTTGCTATAGTAACTTGCT	86-100	8	24	0.75	0.82	0.2802	DY577596
11	WGS051	(GATA)8	F: GCCGAAACTTCACTGGACGA R: AAACTTAACTGAGACAACACAGA	151-216	12	24	0.61	0.86	0.0004	714184394

12	WGS092	(ATT)12	F: CTGGGCAAATATTACCACTTGA R: AAGACAGGTATGTATGCAATGAT	166-184	18	24	0.79	0.93	0.1645	745002572
13	WGS112	(AAT)9	F: ACTCCACTCAGTCCTATTACCA R: ACACTTCCAAGAGTCCCTACA	166-184	6	24	0.79	0.73	0.9619	745001340
14	WGS134	(GATA)6	F: TGTTCGGACCCCAACCTGAT R: GCTGCGCCCTTCGCAATTCA	105-133	7	24	0.58	0.67	0.3767	745001492
15	WGS152	(AT)9	F: GCCTATTTACAATGCATAGCACTA R: CGCTGGGTCCTATCTATATCT	98-118	7	24	0.54	0.76	0.0241	714180564
16	WGS153	(AATC)7	F: TTTCCAAGTTGCTGTGAGTACA R: CGCTGGGTCCTATCTATATCT	106-126	5	24	0.63	0.64	0.9458	714176682
17	WGS189	(ATCT)7	F: AAATGAGCGCCTGTGCACGA R: GAGCATGAAACTCTGAGTAGCA	158-194	9	24	0.58	0.75	0.0420	714180544
18	WGS211	(TAA)8	F: TGACGACGAAACGTTGGCTAT R: AGACCGTTTCCTTTAACCAGAA	181-199	5	24	0.75	0.61	0.4021	714178565

Appendix 2. Example of tags added to the forward primers as a colony ID #1 through #48 for Primer 1, locus EST007.

Primer #	Locus	Microsat	Tag + Primer Sequence	Tag
1	EST007	(TTTC)5	AACACCTGCAATGGTTCTGTTGCAGTCA	AACACC
1	EST007	(TTTC)5	GTTAGGTGCAATGGTTCTGTTGCAGTCA	GTTAGG
1	EST007	(TTTC)5	AACGGATGCAATGGTTCTGTTGCAGTCA	AACGGA
1	EST007	(TTTC)5	AAGAGGTGCAATGGTTCTGTTGCAGTCA	AAGAGG
1	EST007	(TTTC)5	GTTCCATGCAATGGTTCTGTTGCAGTCA	GTTCCA
1	EST007	(TTTC)5	TATGCGTGCAATGGTTCTGTTGCAGTCA	TATGCG
1	EST007	(TTTC)5	TCCTTGTGCAATGGTTCTGTTGCAGTCA	TCCTTG
1	EST007	(TTTC)5	ACAACGTGCAATGGTTCTGTTGCAGTCA	ACAACG
1	EST007	(TTTC)5	TCGACTTGCAATGGTTCTGTTGCAGTCA	TCGACT
1	EST007	(TTTC)5	ACCTCATGCAATGGTTCTGTTGCAGTCA	ACCTCA
1	EST007	(TTTC)5	ACGCAATGCAATGGTTCTGTTGCAGTCA	ACGCAA
1	EST007	(TTTC)5	ACGTGTTGCAATGGTTCTGTTGCAGTCA	ACGTGT
1	EST007	(TTTC)5	ACTCTGTGCAATGGTTCTGTTGCAGTCA	ACTCTG
1	EST007	(TTTC)5	TGACCATGCAATGGTTCTGTTGCAGTCA	TGACCA
1	EST007	(TTTC)5	AGCATGTGCAATGGTTCTGTTGCAGTCA	AGCATG
1	EST007	(TTTC)5	TGGAAGTGCAATGGTTCTGTTGCAGTCA	TGGAAG
1	EST007	(TTTC)5	AGGACATGCAATGGTTCTGTTGCAGTCA	AGGACA
1	EST007	(TTTC)5	TGGTGATGCAATGGTTCTGTTGCAGTCA	TGGTGA
1	EST007	(TTTC)5	ATGTCCTGCAATGGTTCTGTTGCAGTCA	ATGTCC
1	EST007	(TTTC)5	CAACTCTGCAATGGTTCTGTTGCAGTCA	CAACTC
1	EST007	(TTTC)5	CAAGCATGCAATGGTTCTGTTGCAGTCA	CAAGCA
1	EST007	(TTTC)5	CAATGGTGCAATGGTTCTGTTGCAGTCA	CAATGG
1	EST007	(TTTC)5	CACAGTTGCAATGGTTCTGTTGCAGTCA	CACAGT
1	EST007	(TTTC)5	TTGGCATGCAATGGTTCTGTTGCAGTCA	TTGGCA
1	EST007	(TTTC)5	CCATACTGCAATGGTTCTGTTGCAGTCA	CCATAC
1	EST007	(TTTC)5	CCGTTATGCAATGGTTCTGTTGCAGTCA	CCGTTA
1	EST007	(TTTC)5	AGACTTGCAATGGTTCTGTTGCAGTCA	AGACT
1	EST007	(TTTC)5	CCTTCTTGCAATGGTTCTGTTGCAGTCA	CCTTCT
1	EST007	(TTTC)5	CGAGTTTGCAATGGTTCTGTTGCAGTCA	CGAGTT
1	EST007	(TTTC)5	CGTAGATGCAATGGTTCTGTTGCAGTCA	CGTAGA
1	EST007	(TTTC)5	ATCTGTGCAATGGTTCTGTTGCAGTCA	ATCTG
1	EST007	(TTTC)5	CTACCTTGCAATGGTTCTGTTGCAGTCA	CTACCT
1	EST007	(TTTC)5	CTCACATGCAATGGTTCTGTTGCAGTCA	CTCACA
1	EST007	(TTTC)5	CTGAACTGCAATGGTTCTGTTGCAGTCA	CTGAAC
1	EST007	(TTTC)5	CTTGGTTGCAATGGTTCTGTTGCAGTCA	CTTGGT
1	EST007	(TTTC)5	AAGCGTGCAATGGTTCTGTTGCAGTCA	AAGCG
1	EST007	(TTTC)5	GACTTCTGCAATGGTTCTGTTGCAGTCA	GACTTC
1	EST007	(TTTC)5	GAGTCATGCAATGGTTCTGTTGCAGTCA	GAGTCA
1	EST007	(TTTC)5	ATTCCTGCAATGGTTCTGTTGCAGTCA	ATTCC
1	EST007	(TTTC)5	GCACTATGCAATGGTTCTGTTGCAGTCA	GCACTA
1	EST007	(TTTC)5	GCCATTTGCAATGGTTCTGTTGCAGTCA	GCCATT
1	EST007	(TTTC)5	GCTTGATGCAATGGTTCTGTTGCAGTCA	GCTTGA
1	EST007	(TTTC)5	CACCTTGCAATGGTTCTGTTGCAGTCA	CACCT
1	EST007	(TTTC)5	GGATCTTGCAATGGTTCTGTTGCAGTCA	GGATCT
1	EST007	(TTTC)5	GTAACCTGCAATGGTTCTGTTGCAGTCA	GTAACC
1	EST007	(TTTC)5	GTACAGTGCAATGGTTCTGTTGCAGTCA	GTACAG
1	EST007	(TTTC)5	GTCGTATGCAATGGTTCTGTTGCAGTCA	GTCGTA
1	EST007	(TTTC)5	GTGCTTTGCAATGGTTCTGTTGCAGTCA	GTGCTT

Appendix 3. Genotyping workflow.



Appendix 4. Number of alleles, heterozygosity values with standard deviation for datasets random000_len and random000_ID and range of length of microsatellites for each locus.

Locus	# Alleles	Heterozygosity (SD) random000_len	Heterozygosity (SD) random000_ID	Length Range (nt)
Locus 1	2	0.34±0.10	0.35±0.10	28-36
Locus 3	6	0.69±0.07	0.71±0.08	21-36
Locus 4	20	0.91±0.02	0.92±0.02	15-75
Locus 5	11	0.55±0.05	0.63±0.06	57-90
Locus 6	4	0.50±0.11	0.57±0.14	36-45
Locus 8	14	0.83±0.03	0.90±0.03	39-108
Locus 11	8	0.66±0.06	0.79±0.02	12-44
Locus 12	17	0.83±0.05	0.91±0.06	36-93
Locus 13	8	0.72±0.08	0.79±0.10	54-81
Locus 14	12	0.68±0.07	0.74±0.07	20-84
Locus 16	7	0.32±0.24	0.3±0.25	32-56

Appendix 5. Jost's D pairwise differentiation.

	Island						
	Palau			Yap			Ngulu
Site	*S17*	*S20*	*S24*	*S27*	*S29*	*S30*	*S28*
S17	--	0.04	0.09	0.16	0.11	0.16	0.12
S20		--	0.02	0.09	0.05	0.08	0.07
S24			--	0.07	0.04	0.08	0.08
S27				--	0.07	0.03	0.06
S29					--	0.03	0.07
S30						--	0.11
S28							--

Appendix 6. List of 18 primers amplified, modified from Wang et al. 2008.

Primer #	Locus	Repeat motif	Primer sequence (5'-3')	Size (bp)	Allele #	N	No	He	P	Accession #
1	EST007	(TTTC)5	F: TGCAATGGTTCTGTTGCAGTCA R: GATCTCTTTACCGATTTACAGCA	99-107	3	24	0.38	0.47	0.202	DY587595
2	EST014	(TCT)13	F: AGCCGAAGAGGGGACAGAGT R: AGCCGAAGAGGGGACAGACT	143-173	10	24	0.92	0.88	0.693	DY586774
3	EST016	(AAC)7	F: CTATCTGTGTATGATCAGGACTA R: TCCATCTGTTGTGGAAACTGGT	97-122	7	24	0.67	0.69	0.138	DY586537
4	EST032	(TTA)21	F: AGGCACAAGAAAGTGGAAAACAA R: TGAAGGGATGTGAAGCATGGT	138-187	15	24	0.96	0.94	0.727	DY585386
5	EST062	(GAT)9	F: CGAGTTAGTCTGTTAAGATGGT R: CTCTAAGTCCGATCTTCTTCCA	110-126	5	24	0.67	0.71	0.144	DY58448
6	EST097	(TGA)7	F: TGACAACGACATCAATCATGGT R: ACAGCAGGAGCTGTCAGCACT	123-135	5	24	0.71	0.69	0.092	DY583334
7	EST098	(TG)12	F: ACAAATTGCGCTCAAGTTGATG R: ACGGCTGCGAAGGAGTCTAGT	98-118	8	24	0.58	0.68	0.036	DY583314
8	EST181	(ATG)10	F: TGATTGCTGAGAAAGCTAGAGAT R: GCCTCACCTTGCCTTGTACA	145-157	2	24	0.25	0.22	1	DY580714
9	EST196	(TAA)9	F: GTGTTGGCTATCTCATGTATAGT R: ACAACACATCATCAACAACAGCA	117-145	9	24	0.79	0.85	0.093	DY580091
10	EST254	(CA)10	F: GGTGACCAATCAGAGTCTTGA R: TACACTTGCTATAGTAACTTGCT	86-100	8	24	0.75	0.82	0.280	DY577596
11	WGS051	(GATA)8	F: GCCGAAACTTCACTGGACGA R: AAACTTAACTGAGACAACACAGA	151-216	12	24	0.61	0.86	0.000	714184394
12	WGS092	(ATT)12	F: CTGGGCAAATATTACCACTTGA R: AAGACAGGTATGTATGCAATGAT	166-184	18	24	0.79	0.93	0.164	745002572
13	WGS112	(AAT)9	F: ACTCCACTCAGTCCTATTACCA R: ACACTTCCAAGAGTCCCTACA	166-184	6	24	0.79	0.73	0.962	745001340
14	WGS134	(GATA)6	F: TGTTCGGACCCCAACCTGAT R: GCTGCGCCCTTCGCAATTCA	105-133	7	24	0.58	0.67	0.377	745001492
15	WGS152	(AT)9	F: GCCTATTTACAATGCATAGCACTA R: CGCTGGGTCCTATCTATATCT	98-118	7	24	0.54	0.76	0.024	714180564
16	WGS153	(AATC)7	F: TTTCCAAGTTGCTGTGAGTACA R: CGCTGGGTCCTATCTATATCT	106-126	5	24	0.63	0.64	0.946	714176682
17	WGS189	(ATCT)7	F: AAATGAGCGCCTGTGCACGA R: GAGCATGAAACTCTGAGTAGCA	158-194	9	24	0.58	0.75	0.042	714180544
18	WGS211	(TAA)8	F: TGACGACGAAACGTTGGCTAT R: AGACCGTTTCCTTTAACCAGAA	181-199	5	24	0.75	0.61	0.402	714178565

Appendix 7. Pairwise *Jost' D* comparison for 25 sites in Palau (top diagonal) and permutation p values (lower diagonal). In yellow, non-significant *Jost' D* values.

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24	S25
S1	--	0.06	0.01	0.02	0.03	0.03	0.01	0.01	0.03	0.03	0.03	0.06	0.03	0.01	0.02	0.04	0.10	0.07	0.04	0.05	0.03	0.08	0.10	0.02	0.07
S2	0.00	--	0.06	0.08	0.08	0.06	0.06	0.08	0.10	0.09	0.10	0.13	0.07	0.05	0.06	0.09	0.14	0.13	0.10	0.10	0.08	0.14	0.12	0.07	0.11
S3	0.07	0.00	--	0.01	0.04	0.06	0.02	0.05	0.06	0.07	0.07	0.10	0.08	0.06	0.04	0.05	0.14	0.11	0.10	0.10	0.08	0.13	0.15	0.07	0.13
S4	0.01	0.00	0.18	--	0.02	0.04	0.01	0.04	0.05	0.07	0.07	0.12	0.07	0.04	0.06	0.05	0.14	0.09	0.08	0.09	0.08	0.12	0.15	0.06	0.12
S5	0.01	0.00	0.00	0.03	--	0.03	0.01	0.05	0.06	0.06	0.07	0.09	0.10	0.03	0.08	0.07	0.12	0.12	0.08	0.08	0.07	0.12	0.12	0.06	0.10
S6	0.01	0.00	0.00	0.00	0.00	--	0.03	0.02	0.06	0.05	0.05	0.07	0.05	0.01	0.05	0.06	0.10	0.09	0.04	0.05	0.04	0.09	0.09	0.02	0.07
S7	0.09	0.00	0.06	0.15	0.14	0.01	--	0.02	0.04	0.06	0.06	0.09	0.05	0.03	0.03	0.04	0.11	0.08	0.05	0.07	0.04	0.09	0.12	0.03	0.09
S8	0.16	0.00	0.00	0.00	0.00	0.01	0.02	--	0.03	0.02	0.03	0.06	0.02	0.01	0.01	0.03	0.09	0.06	0.02	0.03	0.03	0.07	0.08	0.00	0.06
S9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	--	0.03	-0.01	0.06	0.06	0.04	0.03	0.02	0.04	0.01	0.00	0.01	0.01	0.04	0.06	0.04	0.04
S10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	--	0.03	0.04	0.04	0.03	0.03	0.04	0.07	0.05	0.01	0.02	0.03	0.03	0.05	0.04	0.03
S11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.98	0.00	--	0.04	0.06	0.03	0.03	0.03	0.04	0.02	0.01	0.02	0.01	0.05	0.05	0.04	0.04
S12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	--	0.07	0.08	0.05	0.06	0.05	0.07	0.03	0.05	0.03	0.06	0.06	0.08	0.06
S13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	--	0.03	0.02	0.04	0.10	0.07	0.02	0.03	0.03	0.06	0.06	0.01	0.04
S14	0.07	0.00	0.00	0.00	0.00	0.08	0.00	0.06	0.00	0.00	0.00	0.00	0.00	--	0.04	0.05	0.09	0.07	0.03	0.03	0.02	0.07	0.06	-0.01	0.04
S15	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.00	--	0.01	0.08	0.05	0.02	0.04	0.02	0.06	0.08	0.02	0.07
S16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	--	0.05	0.03	0.03	0.03	0.02	0.06	0.07	0.04	0.05
S17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	--	0.02	0.02	0.03	0.02	0.04	0.03	0.09	0.02
S18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	--	0.00	0.02	0.01	0.03	0.05	0.07	0.03
S19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.21	0.09	0.08	0.00	0.00	0.00	0.01	0.00	0.00	0.12	--	-0.01	-0.02	0.02	0.03	0.02	0.02
S20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.53	--	0.00	0.02	0.02	0.03	0.01
S21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.03	1.00	0.53	--	0.03	0.03	0.03	0.02
S22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	--	0.02	0.07	0.01
S23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	--	0.07	0.00
S24	0.04	0.00	0.00	0.00	0.00	0.04	0.01	0.57	0.00	0.00	0.00	0.00	0.05	0.75	0.01	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	--	0.05
S25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.59	0.00	--

Appendix 8. Matrix of shortest distances in kilometers between sites following the contour of the barrier reef of Palau.

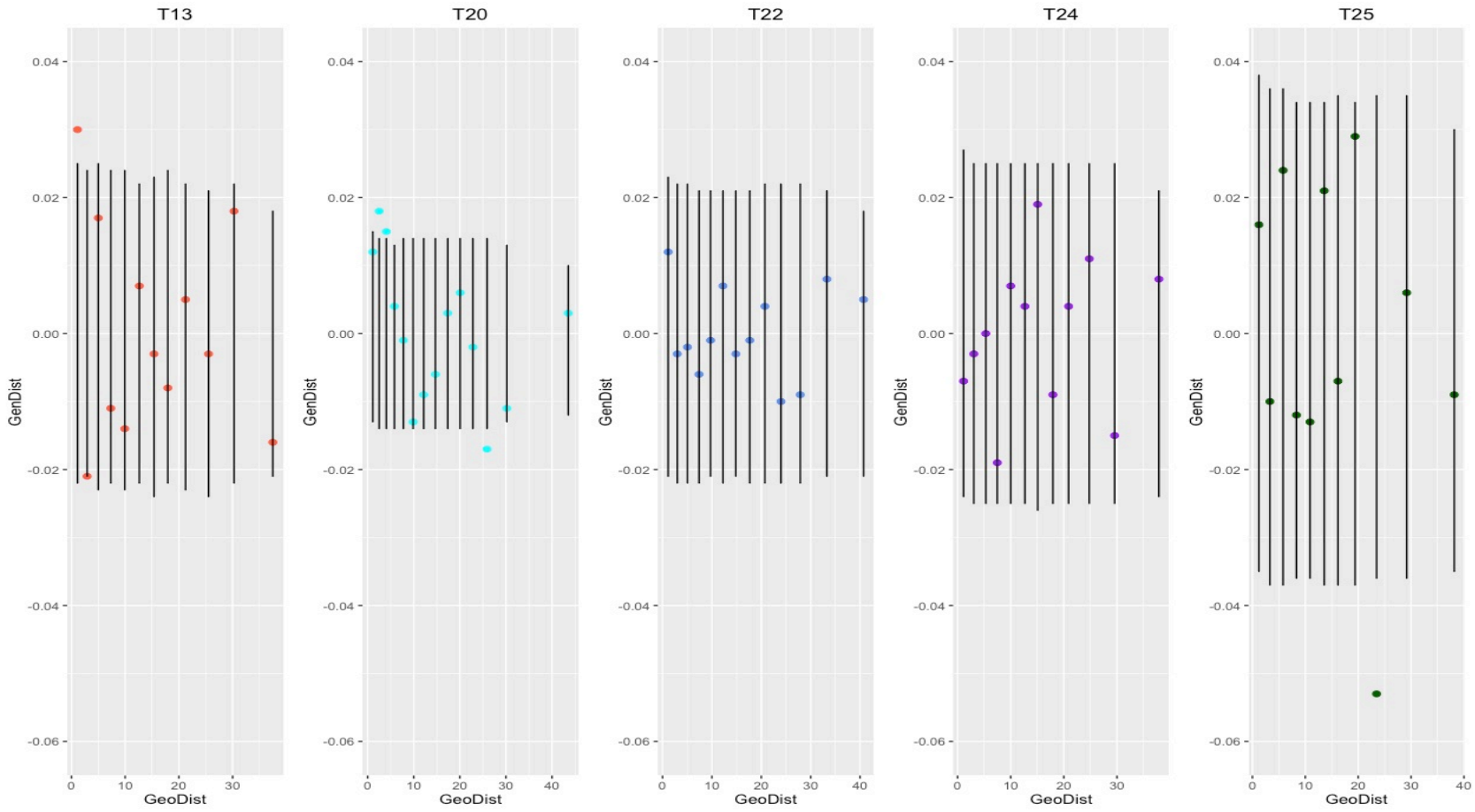
	S21	**S10**	**S23**	**S1**	**S8**	**S9**	**S17**	**S16**	**S15**	**S14**	**S13**	**S12**	**S11**	**S6**	**S20**	**S22**	**S25**	**S19**	**S2**	**S24**	**S3**	**S4**	**S7**	**S18**	**S5**
S21	0	8	16	36	43	57	65	83	92	108	116	131	137	137	151	154	148	137	115	105	90	62	56	28	19
S10	8	0	8	28	34	49	57	74	83	100	107	123	129	129	143	159	157	145	123	114	99	71	64	36	27
S23	16	8	0	20	26	40	49	66	75	92	99	114	121	121	135	151	156	153	131	122	107	79	73	44	35
S1	36	28	20	0	6	21	29	46	55	72	79	95	101	101	115	131	136	148	151	142	127	99	93	64	55
S8	43	34	26	6	0	14	23	40	49	66	73	88	95	95	109	125	130	142	157	148	133	105	99	70	61
S9	57	49	40	21	14	0	8	26	35	51	59	74	81	80	94	110	116	128	149	159	147	119	113	84	75
S17	65	57	49	29	23	8	0	17	26	43	50	66	72	72	86	102	107	119	141	150	156	128	122	93	84
S16	83	74	66	46	40	26	17	0	9	26	33	48	55	54	69	85	90	102	124	133	148	145	139	110	101
S15	92	83	75	55	49	35	26	9	0	17	24	39	46	45	59	76	81	93	114	124	139	154	148	119	110
S14	108	100	92	72	66	51	43	26	17	0	7	23	29	29	43	59	64	76	98	107	123	144	150	136	127
S13	116	107	99	79	73	59	50	33	24	7	0	15	22	21	35	52	57	69	91	100	115	137	143	172	134
S12	131	123	114	95	88	74	66	48	39	23	15	0	7	6	20	36	42	54	75	85	100	122	128	156	149
S11	137	129	121	101	95	81	72	55	46	29	22	7	0	6	14	30	35	47	69	78	93	115	121	150	164
S6*	137	129	121	101	95	80	72	54	45	29	21	6	6	0	6	23	28	40	61	71	86	108	114	143	158
S20	151	143	135	115	109	94	86	69	59	43	35	20	14	6	0	16	22	33	55	65	80	101	108	136	151
S22	154	159	151	131	125	110	102	85	76	59	52	36	30	23	16	0	5	17	39	48	63	85	91	120	135
S25	148	157	156	136	130	116	107	90	81	64	57	42	35	28	22	5	0	12	33	43	58	80	86	115	130
S19	137	145	153	148	142	128	119	102	93	76	69	54	47	40	33	17	12	0	22	31	46	68	74	103	118
S2	115	123	131	151	157	149	141	124	114	98	91	75	69	61	55	39	33	22	0	10	25	46	53	81	96
S24	105	114	122	142	148	159	150	133	124	107	100	85	78	71	65	48	43	31	10	0	15	37	43	72	87
S3	90	99	107	127	133	147	156	148	139	123	115	100	93	86	80	63	58	46	25	15	0	22	28	57	72
S4	62	71	79	99	105	119	128	145	154	144	137	122	115	108	101	85	80	68	46	37	22	0	6	35	44
S7	56	64	73	93	99	113	122	139	148	150	143	128	121	114	108	91	86	74	53	43	28	6	0	29	38
S18	28	36	44	64	70	84	93	110	119	136	172	156	150	143	136	120	115	103	81	72	57	35	29	0	9
S5	19	27	35	55	61	75	84	101	110	127	134	149	164	158	151	135	130	118	96	87	72	44	38	9	0

Appendix 9. Rank of all within site pairwise kinship coefficients and the top 120 between site pairwise kinship coefficients.

Pairs	Mean	Rank
S17	0.0602	1/25
S23	0.0581	2/25
S2	0.0572	3/25
S3	0.0495	4/25
S22	0.0466	5/25
S12	0.0453	6/25
S5	0.0441	7/25
S4	0.0410	8/25
S18	0.0352	9/25
S25	0.0340	10/25
S13	0.0298	11/25
S6	0.0222	12/25
S7	0.0208	13/25
S16	0.0188	14/25
S19	0.0182	15/25
S15	0.0176	16/25
S24	0.0167	17/25
S1	0.0159	18/25
S14	0.0151	19/25
S10	0.0144	20/25
S11	0.0139	21/25
S20	0.0132	22/25
S9	0.0127	23/25
S8	0.0127	24/25
S21	0.0039	25/25
S23_S25	0.0468	1/300
S3_S4	0.0409	2/300
S22_S23	0.0407	3/300
S4_S5	0.0361	4/300
S17_S18	0.0345	5/300
S17_S23	0.0342	6/300
S22_S25	0.0306	7/300
S17_S25	0.0290	8/300
S3_S5	0.0289	9/300
S5_S7	0.0274	10/300

Appendix 10. Pairwise F'_{ST} comparison for 25 sites in Palau (S1 through S25), 3 sites in Yap (S27, S29, S30) and 1 site in Ngulu (S28) (top diagonal) and permutation p values (lower diagonal). In yellow, non-significant F'_{ST} values.

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24	S25	S27	S28	S29	S30
S1	--	0.08	0.02	0.04	0.04	0.04	0.02	0.01	0.05	0.05	0.05	0.10	0.05	0.02	0.03	0.06	0.15	0.11	0.07	0.07	0.05	0.13	0.15	0.02	0.11	0.05	0.14	0.06	0.07
S2	0.00	--	0.09	0.12	0.11	0.09	0.09	0.11	0.15	0.13	0.15	0.19	0.11	0.08	0.09	0.14	0.22	0.20	0.16	0.16	0.12	0.22	0.20	0.11	0.17	0.18	0.23	0.16	0.19
S3	0.09	0.00	--	0.01	0.06	0.09	0.03	0.07	0.10	0.11	0.11	0.16	0.12	0.09	0.07	0.08	0.22	0.17	0.15	0.16	0.12	0.20	0.24	0.10	0.20	0.10	0.21	0.10	0.08
S4	0.02	0.00	0.19	--	0.03	0.06	0.02	0.07	0.08	0.10	0.10	0.18	0.11	0.06	0.09	0.08	0.21	0.14	0.13	0.13	0.12	0.19	0.23	0.08	0.18	0.10	0.18	0.07	0.06
S5	0.01	0.00	0.00	0.03	--	0.05	0.02	0.08	0.09	0.09	0.11	0.14	0.14	0.05	0.12	0.11	0.19	0.18	0.13	0.12	0.11	0.19	0.19	0.09	0.15	0.10	0.20	0.09	0.09
S6	0.00	0.00	0.00	0.00	0.00	--	0.05	0.04	0.08	0.08	0.08	0.11	0.08	0.02	0.07	0.09	0.16	0.14	0.07	0.09	0.06	0.15	0.14	0.03	0.12	0.12	0.18	0.10	0.15
S7	0.09	0.00	0.05	0.13	0.14	0.01	--	0.03	0.07	0.09	0.09	0.14	0.08	0.05	0.05	0.06	0.18	0.12	0.09	0.11	0.07	0.15	0.19	0.05	0.15	0.08	0.16	0.03	0.06
S8	0.14	0.00	0.00	0.00	0.00	0.01	0.03	--	0.05	0.03	0.05	0.10	0.03	0.02	0.01	0.04	0.15	0.09	0.03	0.05	0.04	0.11	0.14	0.00	0.10	0.04	0.09	0.04	0.08
S9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	--	0.05	-0.02	0.09	0.09	0.06	0.05	0.04	0.07	0.02	0.01	0.02	0.02	0.07	0.10	0.07	0.07	0.06	0.10	0.08	0.09
S10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	--	0.05	0.07	0.07	0.05	0.05	0.06	0.11	0.08	0.02	0.04	0.04	0.05	0.08	0.06	0.06	0.08	0.14	0.09	0.11
S11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.98	0.00	--	0.07	0.09	0.05	0.05	0.04	0.07	0.04	0.02	0.04	0.01	0.08	0.09	0.07	0.07	0.07	0.12	0.10	0.12
S12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	--	0.11	0.12	0.08	0.11	0.09	0.11	0.05	0.08	0.06	0.11	0.11	0.12	0.10	0.15	0.22	0.16	0.20
S13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	--	0.04	0.03	0.07	0.16	0.11	0.05	0.06	0.05	0.10	0.11	0.02	0.07	0.12	0.14	0.07	0.14
S14	0.09	0.00	0.00	0.00	0.00	0.11	0.00	0.06	0.00	0.00	0.00	0.00	0.00	--	0.06	0.07	0.14	0.11	0.05	0.05	0.04	0.12	0.11	-0.01	0.07	0.09	0.13	0.06	0.11
S15	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.00	0.00	0.01	0.00	--	0.02	0.13	0.08	0.04	0.07	0.04	0.11	0.14	0.04	0.12	0.07	0.10	0.07	0.12
S16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	--	0.09	0.05	0.06	0.05	0.03	0.11	0.12	0.06	0.09	0.11	0.13	0.10	0.12
S17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	--	0.04	0.05	0.06	0.04	0.08	0.06	0.15	0.05	0.20	0.18	0.17	0.23
S18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	--	0.01	0.03	0.02	0.05	0.10	0.11	0.06	0.12	0.10	0.11	0.13
S19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.24	0.09	0.07	0.00	0.00	0.01	0.02	0.00	0.00	0.16	--	0.00	-0.02	0.04	0.07	0.04	0.04	0.07	0.09	0.07	0.10
S20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.53	--	0.00	0.04	0.04	0.04	0.02	0.10	0.11	0.09	0.12
S21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.03	1.00	0.54	--	0.04	0.05	0.04	0.03	0.08	0.10	0.07	0.12
S22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	--	0.03	0.11	0.02	0.16	0.19	0.13	0.19
S23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	--	0.12	0.00	0.21	0.22	0.19	0.25
S24	0.04	0.00	0.00	0.00	0.00	0.03	0.00	0.53	0.00	0.00	0.00	0.00	0.04	0.73	0.01	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	--	0.08	0.08	0.12	0.06	0.11
S25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.00	0.58	0.00	--	0.17	0.18	0.14	0.19
S27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	--	0.07	0.04	0.02
S28	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	--	0.09	0.14
S29	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	--	0.04
S30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	--

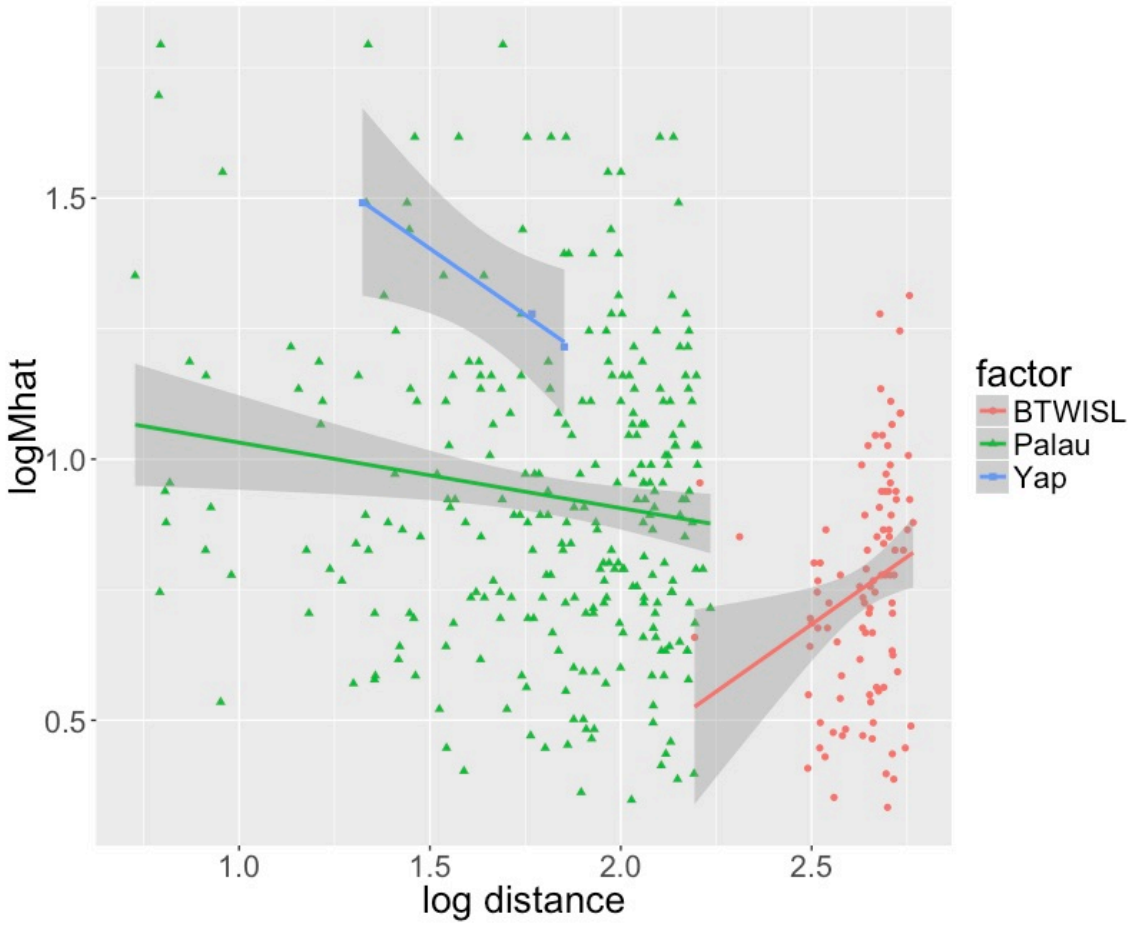


Appendix 11. Autocorrelation of distance and kinship coefficients for colonies in transects T130, T200, T220, T240 and T250 calculated in SPAGEDI. Points outside the 95% CI of the predicted values are significant ($p<0.05$). Distances for geographic distances are in m.

Appendix 12. Dunn post-hoc test displaying the pairwise comparison of difference of mean pairwise kinship coefficients. The p-values are adjusted with the Holm method. In yellow, the non-significant pairwise comparisons.

	Comparison	Z	P.unadj	P.adj
1	YN – PY	15.386	0.000	0.000
2	YN – PN	16.413	0.000	0.000
3	PY – PN	4.160	0.000	0.000
4	YN – Yap	-5.596	0.000	0.000
5	PY – Yap	-22.683	0.000	0.000
6	PN – Yap	-23.459	0.000	0.000
7	YN – among Palau	13.324	0.000	0.000
8	PY – among Palau	-10.296	0.000	0.000
9	PN – among Palau	-11.144	0.000	0.000
10	among Yap – among Palau	20.771	0.000	0.000
11	YN – within Yap	-9.260	0.000	0.000
12	PY – within Yap	-21.831	0.000	0.000
13	PN – within Yap	-22.560	0.000	0.000
14	among Yap – within Yap	-4.677	0.000	0.000
15	among Palau – within Yap	-20.406	0.000	0.000
16	YN – within Palau	-4.899	0.000	0.000
17	PY – within Palau	-40.704	0.000	0.000
18	PN – within Palau	-38.776	0.000	0.000
19	among Yap – within Palau	2.284	0.022	0.045
20	among Palau – within Palau	-38.518	0.000	0.000
21	within Yap – within Palau	7.127	0.000	0.000
22	YN – within Ngulu	-12.935	0.000	0.000
23	PY – within Ngulu	-20.403	0.000	0.000
24	PN – within Ngulu	-20.946	0.000	0.000
25	among Yap – within Ngulu	-9.839	0.000	0.000
26	among Palau – within Ngulu	-19.506	0.000	0.000
27	within Yap – within Ngulu	-6.217	0.000	0.000
28	within Palau – within Ngulu	-11.529	0.000	0.000
29	YN – transect	-7.485	0.000	0.000
30	PY – transect	-50.632	0.000	0.000
31	PN – transect	-46.746	0.000	0.000
32	among Yap – transect	-0.107	0.915	0.915
33	among Palau – transect	-49.265	0.000	0.000
34	within Yap – transect	5.416	0.000	0.000
35	within Palau – transect	-4.222	0.000	0.000
36	within Ngulu - transect	10.475	0.000	0.000

Appendix 13. Plot of gene flow against distance with best fit line. In green, distances between sites in Palau; in blue, distances between sites in Yap; in red distances between sites in Yap, Ngulu and Palau.

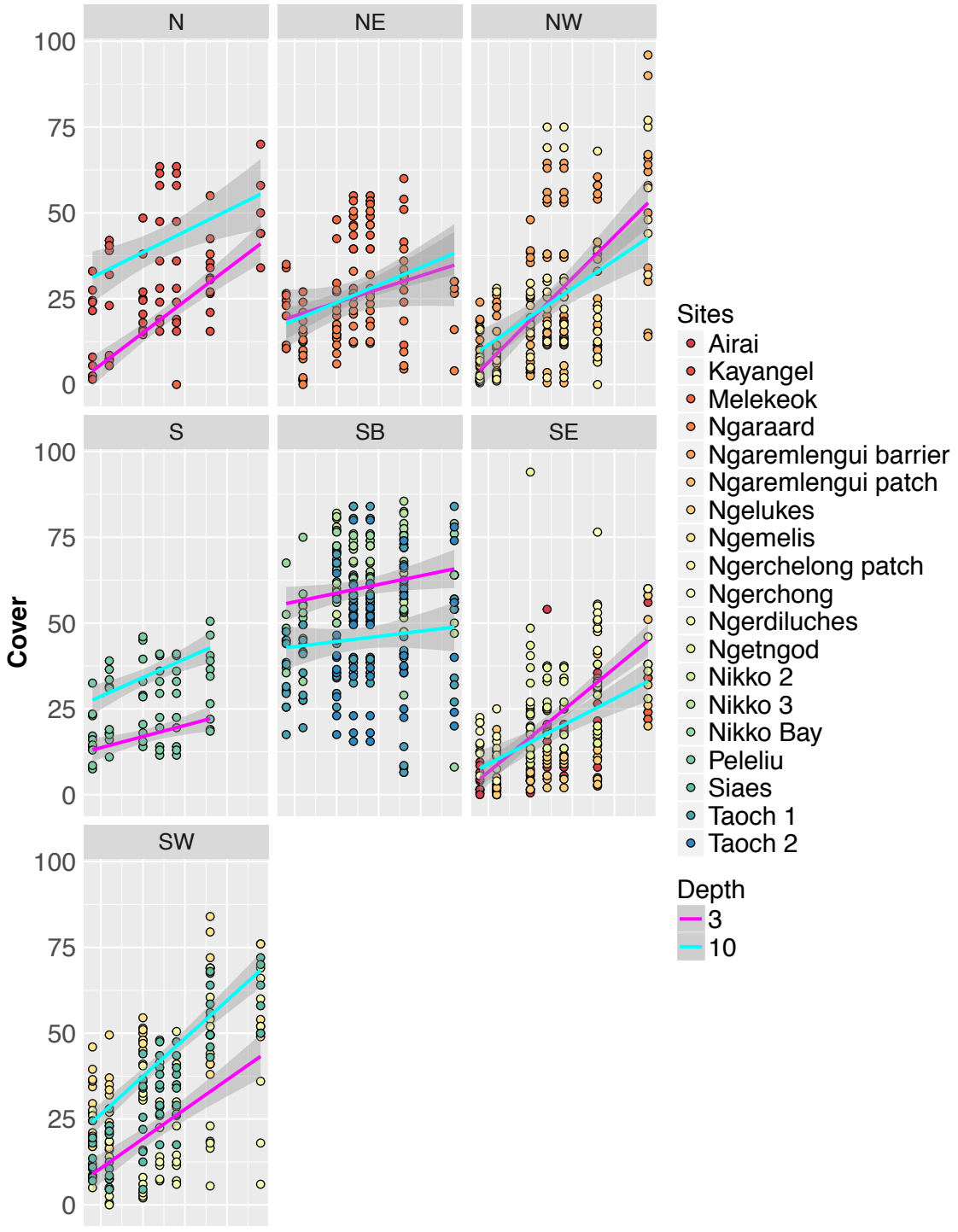


Appendix 14. Monitoring categories abbreviation and classification. The "class" are the abbreviations or names found in the database of Palau International Coral Reef Center. "Pool" are the categories designed for this study.

Class	Pool	Resilience	Class	Pool	Resilience
A	Algae		Echinophyllia	LiveCoral	Susceptible
A.Palifera	LiveCoral	Resistant	Euphyllia	LiveCoral	Intermediate
Acanthastrea	LiveCoral	Resistant	Favia	LiveCoral	Resistant
ACB	LiveCoral	Susceptible	Faviid	LiveCoral	Resistant
ACD	LiveCoral	Susceptible	Favites	LiveCoral	Resistant
Acropora	LiveCoral	Susceptible	Fish	Other	
ACS	LiveCoral	Resistant	Fungia	Other	
ACT	LiveCoral	Susceptible	Fungiid	Other	
Agariciid	LiveCoral	Intermediate	Galaxea	LiveCoral	Resistant
Algae	Algae		Gardinoseris	LiveCoral	Intermediate
Anacropora	LiveCoral	Susceptible	Goniastrea	LiveCoral	Intermediate
Ascidian	Other		Goniopora	LiveCoral	Resistant
Astreopora	LiveCoral	Intermediate	HA	Algae	
Briareum	Other		Halimeda	Algae	
CA	Pavement		Halimeda with mud	Algae	
CARB	Pavement		Heliofungia	Other	
CB	LiveCoral	Susceptible	Heliopora	LiveCoral	Susceptible
CE	LiveCoral	Intermediate	Hydnophora	LiveCoral	
CF	LiveCoral	Intermediate	Hydroid	Other	
CHL	LiveCoral	Susceptible	LC branching	LiveCoral	Susceptible
Clam	Other		LC encrusting	LiveCoral	Intermediate
Clavularia	Other		LC massive	LiveCoral	Resistant
CM	LiveCoral	Resistant	Leaf	Other	
CME	LiveCoral	Susceptible	Leptoria	LiveCoral	Resistant
CMR	Other		Leptoseris	LiveCoral	Intermediate
Coral	LiveCoral	Unknown	Lobophora	Algae	
Coral Unknown	LiveCoral	Unknown	Echinopora	LiveCoral	Intermediate
Coralline Algae	Coralline Algae		Merulina	LiveCoral	Resistant
CS	LiveCoral	Resistant	Millepora	LiveCoral	Susceptible
CSM	LiveCoral	Resistant	Montastrea	LiveCoral	Resistant
Ctenatis	Other		Montipora	LiveCoral	Resistant
CTU	LiveCoral	Unknown	Mussidae	LiveCoral	Susceptible
Cyphastrea	LiveCoral	Resistant	Mycedium	LiveCoral	Resistant
DC	Pavement		Nephthea	Other	
DCA	Pavement		O	Other	
Dichrometra	Other		OT	Other	
Diploastrea	LiveCoral	Resistant	Other	Other	

Class	Pool	Resilience	Class	Pool	Resilience
Oxypora	LiveCoral	Intermediate	Turbinaria	LiveCoral	Intermediate
P	Pavement		Turf algae	Algae	
P. cylindrica	LiveCoral	Intermediate	Unknown Coral	LiveCoral	Unknown
P. rus	LiveCoral	Resistant	ZO	Other	
Pachyseris	LiveCoral	Resistant			
Padina	Algae				
Palauastre	LiveCoral	Unknown			
Pavona	LiveCoral	Resistant			
Pectinia	LiveCoral	Unknown			
Platygyra	LiveCoral	Resistant			
Plerogyra	LiveCoral	Susceptible			
Pocillopora	LiveCoral	Susceptible			
Porites	LiveCoral	Resistant			
Psammocora	LiveCoral	Susceptible			
R	Rubble				
Rubble	Rubble				
S	Sand				
Sand	Sand				
Sandalolitha	Other				
Sarcophyton	Other				
SC	Other				
Anemone	Other				
Sea Ccucumber	Other				
Seriatopora	LiveCoral	Susceptible			
SI	Other				
Sinularia	Other				
Soft Coral	Other				
SP	Other				
Sponge	Other				
Stylaster	LiveCoral	Susceptible			
Stylocoeniella	LiveCoral	Intermediate			
Stylophora	LiveCoral	Susceptible			
Symphillia	LiveCoral	Resistant			
TrachyphilliaA	LiveCoral	Intermediate			
Lobophyllia	LiveCoral				
Lobphytum	Other	Resistant			
MA	Algae				
Trochus	Other				
Tubipora	LiveCoral	Unknown			

Appendix 15. Multiple plots of percent live coral cover over years by exposure for 19 PICRC permanent monitoring sites at two depths. Colours represent different sites. Pink lines are best fit at 3m, turquoise lines are best fit at 10m.



Appendix 16. a. Rank of average percent live coral cover by bleaching susceptibility class, site and depth over years. b. Rank of slopes of linear regression of percent live coral cover by bleaching susceptibility class over years by site and depth.